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THE EFFECTS OF CADMIUM ON RENAL AGING
A Chronic Cadmium Feeding Study in Rats

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16. ABSTRACT

Cadmium (Cd) is known to accumulate preferentially in the renal proximal tubules. Animal and human autopsy studies have shown that damage to the renal proximal tubular cells is associated with toxicity from chronic Cd exposure. The present study was undertaken to determine if Cd exposure influences the natural aging process in the kidney and the accumulation patterns of renal copper and zinc. Male Wistar rats were treated up to 24 months with 0.0, 0.5, 5.0, and 50 mg/l $CdCl_2$ in the drinking water. Every three months, 8 rats from each group were sacrificed to obtain kidneys from which an enriched cortical tubule preparation was extracted. Cd, zinc, and copper concentrations were determined for both the intact cortex and the tubule preparation from each rat. Changes in renal function were assessed by performing urinalysis on a regular basis. Changes in renal structure were assessed by morphometric analysis of fixed kidney sections. The levels of all three metals studied were higher in the tubules than the intact cortex indicating that these metals were concentrated by the tubular cells relative to the other cortical cell types. The toxic effects from Cd exposure were mild and limited primarily to the highest dosed group. The combination of age and Cd dosing had the greatest effect on the accumulation patterns of renal Cd, zinc, and copper. Age alone had the greatest effect on changes in the tissue structure of the kidneys and may have accounted for the decrease in the concentration of renal metals seen in older individuals.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides, and non-ionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

This report describes the results of studies to evaluate the natural aging process in the kidney of rats orally dosed with concentrations of cadmium in water. Concentrations of cadmium, copper, and zinc in the cortical tubules were quantitated to determine whether tubules preferentially accumulate these metals relative to the whole cortex.

F. Gordon Hueter, Ph.D
Director
Health Effects Research Laboratory

ABSTRACT

Cadmium, a nephrotoxin, is known to accumulate in the kidney cortex, preferentially in the renal proximal tubules. Animal and human autopsy studies have shown that the kidney is the critical organ and that damage to the renal proximal tubular cells is associated with toxicity due to chronic cadmium exposure. Several investigations have shown an age-associated change in the levels of renal cortical cadmium resulting from chronic exposure in laboratory animals and long-term environmental exposure in humans.

The present study was undertaken to determine if cadmium exposure influences the natural aging process in the kidney. Also, the effect of cadmium dosing on the accumulation patterns of renal copper and zinc was investigated. Further, the concentrations of cadmium, copper and zinc in the cortical tubules were quantitated to determine if the tubules preferentially accumulate these three metals relative to the whole cortex.

In this study, 272 male Wistar rats were treated up to 24 months with 0.0, 0.5, 5.0 and 50 mg/l cadmium, as $CdCl_2$, in the drinking water. Every three months, 8 rats from each group were sacrificed to obtain kidneys from which an enriched cortical tubule preparation was extracted. Cadmium, zinc and copper concentrations were determined for both the intact cortex and the tubule preparation from each rat. Changes in renal function were assessed by performing urinalysis on a regular basis. Changes in renal structure were assessed by morphometric analysis of fixed kidney sections. The levels of all three metals studied were higher in the tubules than the intact cortex, on a dry weight basis, indicating that these three metals were concentrated by the tubular cells relative to the other cortical cell types. The concentration of cadmium in both the cortex and tubules increased with dose level and duration of exposure. This increase was linear up to 12 months in the two low dose groups and up to 15 months in the highest dose group. After 15 months, the levels of cadmium decreased in both the tubules and the whole cortex. Zinc and copper concentrations in the cortex and tubules were significantly evaluated by cadmium exposure, with the greater effect on copper. Zinc and copper levels in the tubules also decreased significantly after 15 months.

The toxic effects from cadmium exposure were mild and limited primarily to the highest dosed group. The combination of age and cadmium dosing had the greatest effect on the accumulation patterns of renal cadmium, zinc and copper.

Age alone had the greatest effect on changes in the tissue structure of the kidneys and may have accounted for the decrease in the concentration of renal metals seen in older individuals. Age alone also had the greatest effect on the physiological functioning of the kidney as measured by proteinuria, decreased urine and renal leucine aminopeptidase activity, changing diurnal urine volumes and decreased urine pH and specific gravity.

The combination of old age and high cadmium dosing appeared to affect renal functioning as indicated by increased urine volume, increased urine glucose levels and decreased specific gravity.

Increased cadmium dosing appeared to be associated with certain structural changes in the kidney. Although not significant, the highest dosed animals had a greater prevalence of moderate to severe tubular hyperplasia and tubular dilation/cast formation. The highest dosed rats also had significant larger kidneys as measured by their percent of the total body mass.

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SECTION 1

INTRODUCTION

Cadmium, a non-essential trace element used in many industrial processes and consumer products, is now widely distributed throughout the environment. This metal may be present as a contaminant in air, food, water and cigarette smoke. There is increasing concern over the possible health effects from chronic, low-level exposure to cadmium because of the steadily increasing world-wide use and distribution of this metal. In nature, cadmium is usually found in close association with zinc, a trace element having similar physical and chemical properties. Cadmium is obtained as a by-product of zinc production. Used industrially for about 100 years, cadmium is used as a paint pigment, as a corrosion resistant plating for steel, as a stabilizer for plastics, and as a component for batteries. It also is used in alloy manufacture, electronics, and nuclear engineering.

In contrast to zinc, which is essential for normal human growth and development, cadmium is biologically nonessential and toxic. In an occupational setting, chronic exposure to cadmium is through the inhalation of cadmium dusts or fumes. In an environmental setting, exposure is through ingestion of contaminated food or water. The fraction of the exposure dose that is absorbed by the lungs or the gastrointestinal tract is very efficiently retained in the body. Because only a very small portion of the body burden actually is excreted, cadmium has an estimated biological half-life of 10 to 40 years in man (Kazantzis, 1979a). Absorbed cadmium is stored mainly in the liver and kidneys.

In long-term, low level exposures, such as those associated with background environmental levels of cadmium, accumulation in the kidney will occur over much of the individual's life span. Several investigations (Gross et al., 1976; Travis and Haddock, 1980) have shown that in non-occupationally exposed populations, cadmium concentrations in the kidney cortex increase linearly from birth, peak around fifty years of age, and decline thereafter. About half the total body burden is in the liver and kidneys combined and about one-third is in the kidneys alone.

The renal cortex is the critical organ in chronic, low-level exposures. In the cortex, cadmium is concentrated in the epithelial cells of the proximal tubules, in the form of a cadmium-metallothionein complex. It has been estimated that a concentration of 200 μg Cd/g wet weight of cortex is the threshold level for renal damage (Friberg et al, 1974).

Many animal studies (Kawamura, et al., 1978; Nishizumi, 1975; Itokawa, et al., 1974; Kawai, et al., 1976) have shown that chronic cadmium ingestion is associated with renal morphological changes including: (1) proximal convoluted tubule cell damage ranging from epithelium atrophy to degeneration and necrosis of the cells, and (2) interstitial cell damage ranging from slight edema to fibrosis and interstitial nephrosclerosis. Studies involving rats and rabbits (Itokawa, et al., 1974; Nomiyama, 1974; Kawai, et al., 1976) have shown that cadmium also can induce marked degeneration of renal glomeruli.

As with human autopsy studies, some chronic cadmium feeding studies in animals (Perry et al., 1974; Nomiyama, 1974) have shown a definite time-dependent pattern of renal cadmium accumulation as a function of age. Similar to the situation in humans, renal cadmium concentrations in these animals correlate positively with age and increasing cadmium dose in the young and middle aged individuals; however, the concentrations decrease with old age.

Because of this documented age-associated change in renal cadmium levels, the present study was designed to examine the effects of natural aging and cadmium exposure on the tissue structure and physiological functioning of the kidney. The primary hypothesis to be tested was whether cadmium exposure influences the physiological, morphological, and biochemical aging of the kidney.

Since cadmium is thought to accumulate preferentially in renal proximal tubules, it was desirable to separate out the tubules and relate the physiological changes in the kidney to the cadmium levels in the tubules. Traditionally, the renal cadmium burden has been evaluated as the concentration of cadmium in the intact cortex; therefore, a comparison of the levels of metals in the tubules with that of the cortex was needed to determine if the cortex levels were good indicators of tubular levels. It was hoped that by separating out the tubules, it would be possible to gain a more accurate picture of the relationship between renal cadmium accumulation and renal toxicity. Thus, a secondary hypothesis being tested was whether cadmium concentrations in the renal cortex are sufficient to predict the cadmium dose to the renal tubules.

Since tissue accumulation of cadmium is closely associated with that of zinc and copper, additional hypotheses to be tested were whether the renal tubules preferentially concentrate zinc and copper and if cadmium affects the accumulation of these two metals.

SECTION 2

BACKGROUND

REVIEW OF RENAL PHYSIOLOGY

The renal system plays a very important role in the maintenance of the body's homeostasis. To accomplish this, the kidney has a number of functions, including the excretion of toxic materials and metabolic waste products, and the regulation of the chemical composition, volume and pH of the body's extracellular fluid. The kidneys conserve body water and the major electrolytes, especially sodium, potassium, bicarbonate and chloride ions. However, the kidneys also eliminate excess water, electrolytes, and osmotically active substances resulting from fortuitous influxes. The kidneys function in the endocrine system by producing hormones, such as erythropoietin, which stimulates the production of red blood cells in the bone marrow. Thus, anemia is one clinical manifestation of chronic renal failure, which causes the reduction of total renal mass and consequent marked reduction in erythropoietin production (Tuttle and Schottelius, 1969; Koushanpour, 1976).

The functional unit of the kidney is the nephron (Fig. 2-1). There are approximately 1.25 million nephrons in the adult human kidney. The nephron has two major components: 1) a renal corpuscle or ultrafilter, comprising a tuft of glomerular capillaries and Bowman's capsule, and 2) a renal tubule or tubular exchanger that is divided into the functional segments of proximal tubule (including convoluted and straight segments), loop of Henle, distal tubule (including straight and convoluted segments), and collecting duct. As illustrated in Figure 2-1, each zone of the kidney contains particular segments of the nephron. In general, the outermost zone, the cortex, contains the glomeruli, the greater proportion of the proximal and distal tubules and the beginning of the collecting ducts. The outer medulla contains the thin descending and thick ascending limbs of the loop of Henle and the collecting ducts. The inner medulla contains the thin descending and ascending limbs of the loop of Henle and the larger collecting ducts (Tuttle and Schottelius, 1969; Moffat, 1971; Koushanpour, 1976).

Vital to proper renal functioning, an elaborate renal vascular system provides a network for the rapid and extensive exchange of materials between the tubules and the blood. The renal artery subdivides into interlobar arteries, which in turn give rise to arcuate arteries. The arcuate arteries divide into a network of intralobular arteries. The vas afferens, a branch of an intralobular artery, enters Bowman's capsule and breaks up into a large number of capillaries to form the glomerulus. The capillaries then reunite to give rise to the vas efferens, the arteriole that leaves the capsule. Soon

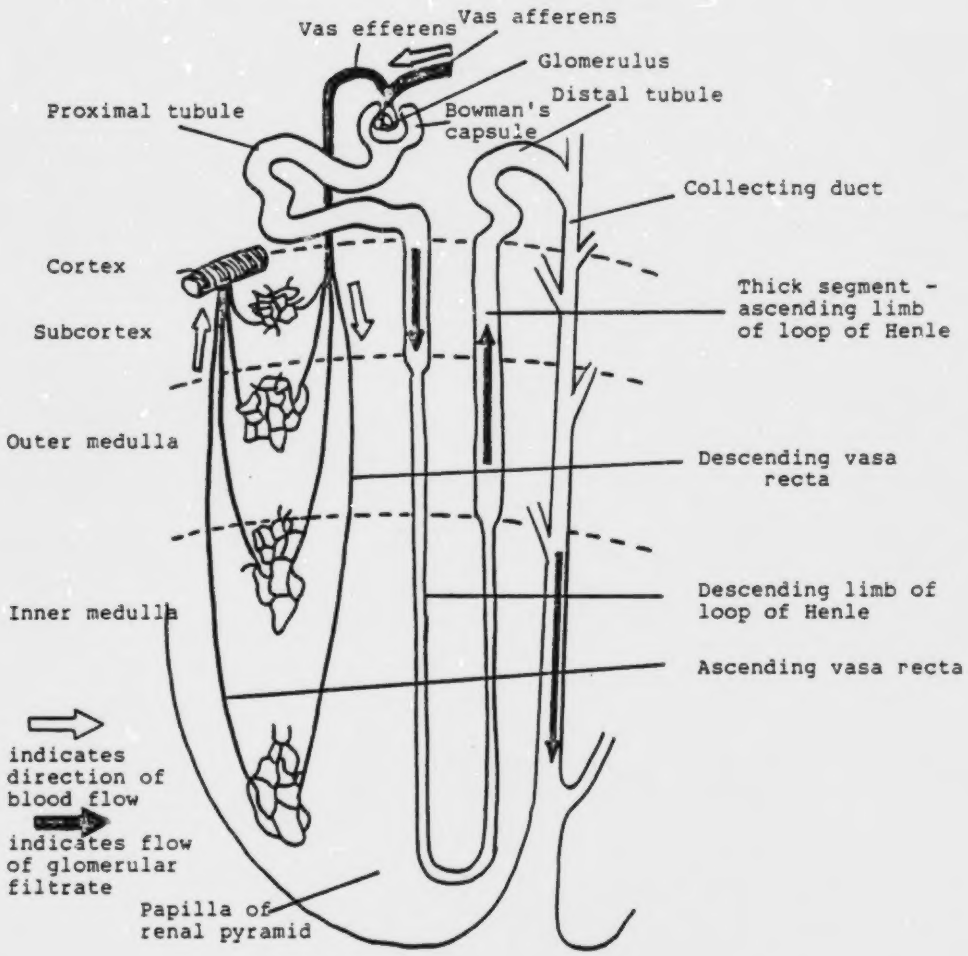


Figure 2-1. Schematic Diagram Illustrating the Different Segments of the Nephron and the Zones of the Kidney in Which They Lie

(Adapted from Moffat, 1971).

after leaving the capsule, the vas efferens break up into a second capillary bed, the peritubular capillaries, which surround the proximal and distal convoluted tubules. Associated with the juxtamedullary glomeruli, an additional capillary bed, the vasa recta, is formed by long hairpin loops of vessels that extend alongside the loops of Henle into the inner medulla. These capillaries eventually combine to form venules, which in turn become interlobular veins, interlobar veins and exit the kidney as the renal vein (Tuttle and Schottelius, 1969; Moffat, 1971; Koushanpour, 1976).

Normal functioning of the nephron consists of two processes:

1. The glomerulus produces a large (approximately 180 1/24 hours in man), essentially protein-free ultrafiltrate of blood plasma. The glomerulus normally is permeable to all other plasma constituents (e.g., water, glucose, salts, nitrogenous waste products) except the large blood proteins, such as albumin. Thus, the filtrate contains all the crystalloids in the same osmolar concentration as that in the aqueous phase of the plasma (Tuttle and Schottelius, 1969; Koushanpour, 1976).

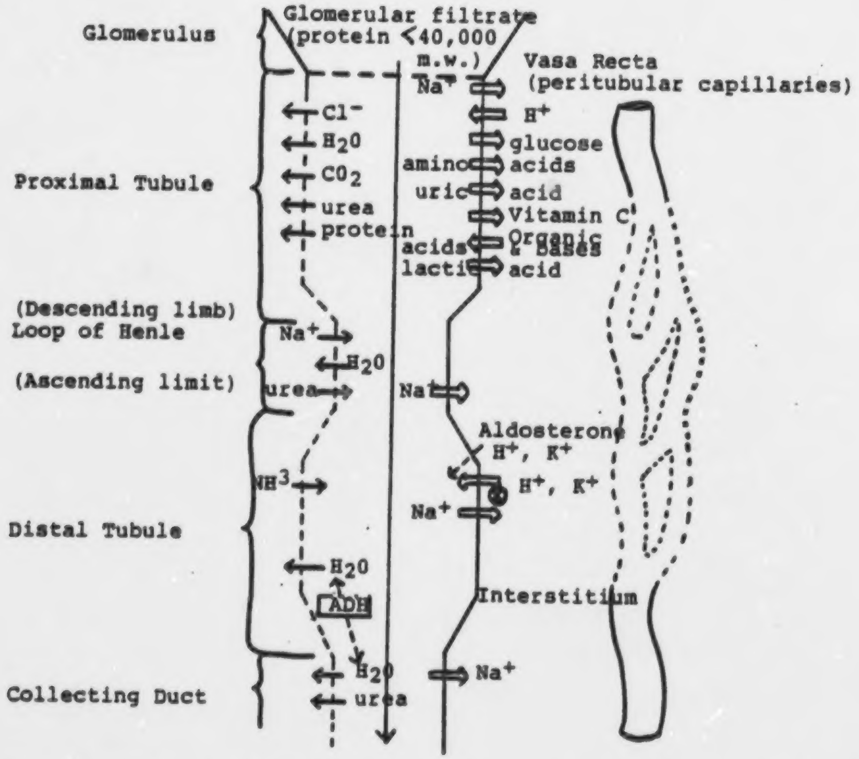
2. The renal tubules selectively process the ultrafiltrate Approximately 99% of the filtered water (178.5 1/24 hours in man) is conserved, thereby leaving about 1.5 1/24 hours to be excreted in the urine. The tubules selectively conserve filtered substances by two exchange processes. In one process secretion, the lining cells of the tubule move substances from the surrounding intersitium and into the intratubular filtrate. In the other process, reabsorption, substances are moved from the tubular filtrate, across the tubular cells and back into the renal interstitium (Bulger, 1977; Tuttle and Schottelius, 1969; Koushanpour, 1976).

The renal tubules selectively conserve the filtered water and osmotically active substances, thereby adjusting the osmolality of the urine to suit the body's needs. In man, maximally concentrated urine may have an osmolality that is four to five times greater than the osmolality of the blood plasma and, therefore, the glomerular filtrate (Koushanpour, 1976).

Each segment of the renal tubule has a different cellular structure and performs a different function in terms of the substances it absorbs and/or secretes. Figure 2-2 summarizes the transport of materials into and out of each section of the renal tubule. The large volume of dilute glomerular filtrate is reduced by passive diffusion of water out of the tubules and collecting ducts in response to an osmotic gradient. This movement of water is directly linked to the movement of solutes such as sodium. Some solutes move into or out of the tubules along their chemical or electrical gradients, while others are transferred by energy-dependent active transport mechanisms (Tuttle and Schottelius, 1969).

The proximal tubule reabsorbs approximately 80% of the volume of water from the glomerular filtrate. This reabsorption is relatively constant. The remaining 20% enters the loop of Henle and is reabsorbed to varying degrees along the remainder of the nephron. Experiments using micropuncture tech-

Figure 2-2. Summary of Renal Tubular Reabsorption and Secretion



Active transport mechanisms are indicated by ⇨, passive diffusion by ⟶ , and exchange pumping by ⊗ . ADH = antidiuretic hormone.

(From Tuttle and Schottelius, 1969).

niques to determine the composition of tubular fluids have shown that reabsorption of water in the proximal tubule is secondary to the active reabsorption of sodium. Despite the absorption of various substances into the proximal tubule (Fig. 2-2), the fluid within this section of the nephron remains iso-osmotic with respect to plasma so that there is no concentration or dilution of the tubular fluid in this region (Tuttle and Schottelius, 1969; Moffat, 1971; Bulger, 1977).

The mechanisms for actually concentrating the tubular fluid reside in the loop of Henle, distal convoluted tubules, and collecting ducts. The process is facilitated by an osmotic gradient that exists in the tissues of the medulla as a result of the active transport of sodium. The osmolality increases significantly from the outer medulla toward the papilla. The osmolality of fluids in all structures (e.g., tubules, blood vessels, interstitium) at any one level in the medulla is approximately equivalent. Because of this osmotic gradient and the well documented process of counter current multiplication, the iso-osmotic fluid leaving the proximal tubule loses water and becomes increasingly concentrated as it descends the loop of Henle. As the fluid comes up the ascending limb, it loses salts and becomes less concentrated, until it is hypo-osmotic as it enters the distal convoluted tubule. At this point, the body's need to conserve water will dictate whether a large volume of dilute urine or a small volume of concentrated urine is produced. This step is facilitated by circulating levels of pituitary anti-diuretic hormone (ADH), which respond to the need to conserve water, and acts by controlling the water permeability of the distal convoluted tubules and collecting ducts. Thus, if the body needs to conserve more water, the levels of ADH rise, causing increased tubular permeability and water reabsorption in this portion of the nephron and resulting in the production of small volumes of concentrated urine (Tuttle and Schottelius, 1969; Moffat, 1971; Koushanpour, 1976).

The renal system interacts with the pulmonary and blood buffer systems to maintain the acid-base balance of the body fluids. To accomplish this, the kidneys maintain the total quantities and concentrations of the major electrolytes within normal limits by a) stabilizing the standard bicarbonate pool through absorption of the filtered bicarbonate and b) excreting an appropriate amount of the fixed acids that are produced by metabolism. The kidneys have the ability to excrete acid or alkali selectively in the urine because of an ion-exchange mechanism which operates in the proximal and distal tubules and the collecting ducts. In man, the kidneys cannot produce urine with a pH below 4.5, while the maximum alkalinity is around pH 8 (Tuttle and Schottelius, 1969; Koushanpour, 1976; Becker et al., 1971).

Normally, 80 to 85 percent of the filtered bicarbonate is reabsorbed in the proximal tubule and the remainder is reabsorbed in the distal tubules and collecting ducts. The absorption of ions in the proximal tubule results in minimal acidification of the tubular fluid, and therefore, there is no net excretion of H^+. Urine is acidified (and H^+ eliminated) in the distal tubules and collecting ducts through the formation of free titratable acids, such as NaH_2PO_4, and through the tubular synthesis of ammonia and the excretion of acidic NH_4Cl (Tuttle and Schottelius, 1969; Koushanpour, 1976; Becker et al., 1971).

As indicated in Figure 2-2, all constituents of the glomerular filtrate, except water, sodium, urea, and bicarbonate are handled almost exclusively in the proximal tubules. Since Cd exposure can result in damage to the proximal tubules and increased urinary excretion of proteins, glucose, and amino acids (Tsuchiya, 1978; Friberg et al., 1974; Nomiyama et al., 1973), the normal renal handling of these materials has been reviewed.

As noted previously, the glomerular filtrate is practically protein-free; however, even under normal conditions, a small amount of protein (40-80 mg/day in man) may be excreted in the urine. This includes excretion of albumin and globulins (Koushanpour, 1976). The types of proteins appearing in the glomerular filtrate depend, to a large extent, on the size of the protein. Thus, the glomerular capillaries are permeable to many low molecular weight proteins (molecular weight under 40,000) including β_2-microglobulin, immunoglobulin light chains, small serum enzymes, and hemoglobin. Approximately thirty different plasma protein components have been identified in the urines of healthy individuals (Peterson et al., 1969; Mogielnicki et al., 1971).

In man, virtually all the filtered albumin is reabsorbed in the proximal tubules by an active transport process. The reabsorptive mechanism of the tubules becomes saturated at plasma concentrations of 6-7 g% (Koushanpour, 1976). One model to explain the normal renal handling of filtered proteins involves their uptake by pinocytosis into the cells of the proximal convoluted tubules. The proteins are catabolized, and the resultant amino acids are reabsorbed into the surrounding peritubular capillaries. In this situation, only a very small amount of the filtered protein actually appears in the urine (Mogielnicki et al., 1971; Ham, 1974).

Glucose is freely filtered by the glomerulus. Micropuncture studies in rats have shown that virtually all the filtered glucose is reabsorbed in the proximal tubules. Except for trace amounts, glucose is not present in normal urine. An active, carrier-mediated transport system is involved in the movement of glucose from the tubular lumen to the surrounding peritubular capillaries. Glucose appears in the urine (glycosuria) when the filtered load of glucose exceeds the reabsorptive capacity of the tubules. Once this capacity has been reached, increases in urinary glucose are proportional to increases in plasma glucose (Koushanpour, 1976; Becker et al., 1971).

Most amino acids are reabsorbed in the proximal tubules by an active, sodium-dependent process similar to that for the reabsorption of glucose. Carrier-mediated transport is involved so that the tubules have a limit to their reabsorptive capacity. There appear to be at least four separate structure-specific transport systems. Each system handles one of the following groups of amino acids: neutral amino acids, basic amino acids, acidic amino acids, and iminoglycine amino acids. Normally, because the tubular reabsorptive capacity for most amino acids appears to be very high, all filtered amino acids are completely reabsorbed and none appear in the urine (Koushanpour, 1976).

NORMAL RENAL AGING IN THE RAT

The rat has been used as a model in several studies to determine the effect of aging on the kidney (Lowenstein, 1979; Snell, 1967; Anver and Cohen, 1979;

Berg, 1965; Arataki, 1926; Andrew and Pruett, 1957; Sworn and Fox, 1974). Renal structure, function, and metabolism all are altered during aging. Several variables affect the development of age-associated lesions in the rat kidney and these variables must be considered when interpreting data and making comparisons between different studies. Factors such as husbandry, intercurrent infectious disease, nutrition, breeding status, and genetic background can alter the age of onset, severity and type of renal lesion. As an example, obese rats are more susceptible to chronic nephritis (Snell, 1967).

Histological studies in both man and animals have demonstrated that gradual morphological changes occur in the normal kidney over the entire life-span of the animal. These changes include a loss of nephrons with increasing age and changes in the glomerulus, tubules, and interstitium. Four main categories of histological changes commonly found in the senile human kidney are: 1) glomerular changes, ranging from congestion to fibrosis or hyalinization, 2) tubular changes, particularly dilation and atrophy, 3) vascular changes, particularly of the arterioles, and 4) interstitial changes, mainly sclerosis of cortical connective tissue. For all of these changes, there is a large interindividual variation of the severity and proportion of renal mass involved (Andrew and Pruett, 1957).

Several studies have shown that in the rat, the total weight of the kidney, the number of glomeruli and nephrons, and size of the glomeruli change as a function of age. Using normal Wistar Institute rats, Arataki (1926) showed that the kidneys increased in weight up to age 150 days. Between 200 and 350 days, the weight fluctuated somewhat (between 97 and 80 percent of the peak value at 150 days). By 500 days, the weight had decreased by 33 percent of the peak value. It should be noted that the sample size used by Arataki was very small, only one rat per sacrifice, and that the study ended at 500 days. Considering the wide interindividual variations in organ weights, it is not possible to determine from these results if the senile rat kidney actually becomes smaller. In contrast, other researchers (Andrew and Pruett, 1957; Berg, 1965) have found that the kidney weight continues increasing with age at least through age 1,170 days.

Arataki (1926) assessed changes in the number of nephrons in the Wistar rat by enumerating the fully formed glomeruli. The glomeruli undergo an early proliferative phase where the number increases rapidly, followed by a stationery phase where the number remains fairly constant, and then end in a declining phase where the number rapidly decreases. The mean value of glomeruli for both sexes is about 10,500 at birth. The maximum of 30,000 was reached at about 100 days and remained stable through the age of 350 days. By age 500 days, the number of glomeruli dropped to approximately 22,600 which was a 25% decrease compared to the peak value. Since the size of the kidney and the number of glomeruli change as a function of age, the glomerular numerical density also changes with age. Thus the number of glomeruli per cubic millimeter of cortex was at its peak of about 1,000 at birth and then fell sharply to a value of 68 by 90 days. This value, which represents a 93.2% reduction from the peak value, remained fairly constant through 500 days.

The number of glomeruli in the normal human kidney also varies as a function of age. By counting the glomeruli from kidneys of several differently aged individuals, Moore (1961) concluded that man, unlike the rat, does not undergo a phase of postnatal nephrogenesis in which the number of glomeruli increases after birth. He found that from birth to age forty years, the kidney contains roughly 800,000 to 1,000,000 glomeruli. Analogous to the rat kidney, he found that the human kidney also loses about one-third to one-half of the glomeruli by age seventy years.

There are many well characterized tissue changes associated with normal renal aging in the rat, although the onset and severity of such changes vary among the different rat strains. Chronic renal disease, a significant cause of morbidity and mortality in many rat strains, has several synonyms: chronic nephritis, chronic nephrosis, chronic progressive nephrosis, glomerulosclerosis, glomerulonephritis, and nephropathy. Severely affected end-stage kidneys, as often seen in rats over one year of age, have the following characteristics: 1) the glomeruli have eosinophilic deposits and thickened basement membranes in the capillary tufts and Bowman's capsules, 2) adhesions exist between the parietal and visceral layers of Bowman's capsule, 3) the glomerulus has partial to complete hyalinization or sclerosis, 4) some tubules are dilated with flattened epithelium and proteinaceous casts in the lumens, while other tubules are collapsed and atrophic, 5) the cortical interstitium is fibrotic and infiltrated by lymphocytes and other mononuclear cells (Anver and Cohen, 1979; Andrew and Pruett, 1957).

Pathogenic changes begin at an early age in the rat and consist of glomerular lesions. Generally, in rats three to six months old, there is a PAS-positive thickening of the glomerular basement membrane in a few scattered glomeruli (Anver and Cohen, 1979). In addition to these early glomerular changes, Berg (1965), studying male Sprague-Dawley rats, found a few dilated proximal convoluted tubules containing casts. These dilated tubules were found adjacent to the affected glomeruli.

These nephrotic lesions occur more frequently with increasing age. Coleman *et al*. (1977), studying Fischer 344 male rats from four to 33 months of age, found a highly significant correlation between increased age and both the incidence and severity of chronic nephropathic changes.

By 12 to 14 months of age, a large proportion of individuals have proteinuria greater than 2.0 mg/ml. Glomerulosclerosis apparently develops following the onset of proteinuria. Initially, glomerulosclerosis is focal, but later becomes more diffuse and involves partial to complete obliteration of the glomerular tufts. Thus, it appears that chronic nephritis in the rat starts with lesions in the glomerulus and then spreads to involve the tubules (Anver and Cohen, 1979; Berg, 1965). Hirokawa (1975), working with Wistar rats, theorized that tubular and interstitial lesions were secondary to and caused by excessive leakage of protein through the diseased glomeruli.

In contrast, describing the onset and progression of renal lesions in the rat, Snell (1967) indicated that pathological changes start in the tubules and that the glomeruli are the last element of the nephron to be affected. In her review of several NIH inbred rat strains, including the Wistar, Snell noted that in moderately severe chronic nephritis, only an occasional glomerulus may be mildly affected. Further, even in the most severely damaged kidney, a few relatively normal glomeruli are still present. Snell felt that differences among the various rat strains explain why several investigators found glomerular lesions to be prominent, while she found the tubular lesions to be prominent.

Andrew and Pruett (1957), studying kidneys of male Wistar rats aged 2.5 months to 3.2 years, reached the same conclusion as Snell (1967) on the prominence of tubular lesions. Further, these investigators found that the most significant glomerular change of senile rats was the thickening of the basement membrane and dilation of the glomerular capillaries.

There appear to be limitations in using the rat as a model for renal aging in man. There are, however, several similarities between the aging rat kidney and the aging human kidney. Humans and rats are similar in that renal function and the number of nephrons diminish with age (Lowenstein, 1979). Also, similar tubular and glomerular changes have been observed in senescent rat and human kidneys. It has been noted that severe chronic nephritis in rats presents most of the features of human glomerulonephritis except for the lack of vascular damage, and the secondary contraction of the kidney due to fibrosis (Snell, 1967).

There are certain dissimilarities in renal aging between rat and man. Several investigators (Snell, 1967; Lowenstein, 1979; Sworn and Fox, 1974; Andrew and Pruett, 1957) have found that blood vessels in the rat kidney are resistant to the development of arteriosclerosis, arteriolosclerosis or occlusion. In contrast, nephrosclerosis may play a dominant role in the reduced renal function of elderly humans.

Whereas renal senescence in man is believed to result from alterations in the renal arterial system, it is suggested that age-related changes in the rat kidney are the result of misdirected hyperplasia of the tubular epithelium. This hyperplasia results in tubular blockage, proximal tubular dilation and eventual glomerular sclerosis (Snell, 1967; Sworn and Fox, 1974). Such observations suggest that the mechanisms of renal aging in rodents differ from those in the human and that using the rat as a model for renal aging in man must be done with caution (Sworn and Fox, 1974; Andrew and Pruett, 1957).

CADMIUM ACCUMULATION AND DISTRIBUTION

The effects of Cd poisoning have been reviewed in several documents, including Friberg _et al_. (1974; 1977), Tsuchiya (1978), Flick _et al_. (1971), Webb (1979), and Nriagu (1981). Cd accumulates primarily in the kidney and liver, with smaller amounts distributed in the pancreas, lungs, spleen, testes, bone, blood and other tissues.

Cd is toxic to virtually every organ system in the animal body. The tissue distribution of Cd, the accumulated concentration of Cd in the tissues, and the resulting sites and extent of cellular damage vary considerably depending on the route of Cd administration, the type of Cd compound, the magnitude of the dose, the duration of exposure, the age, sex and nutritional status of the individual, and in the case of animals - the species (Friberg _et al_., 1974; Webb, 1979; Samarawickrama, 1979).

Figure 2-3 presents one plausible flow scheme depicting the movement of Cd between the different compartments of a system. The construction of this diagram was based on data from experimental animal exposures and from human exposures in both industrial and environmental settings. The diagram was used to construct a kinetic model for describing the metabolism of Cd and calculating the daily Cd intake necessary to reach the critical kidney cortex concentration of 200 μg/g wet weight (Kjellstrom and Nordberg, 1978).

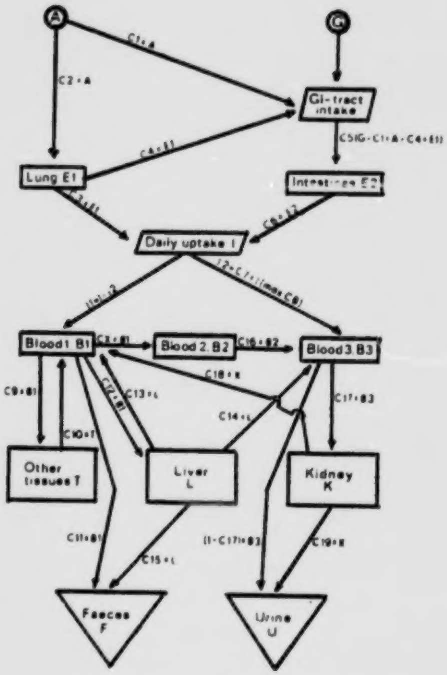
Cd enters the body primarily through inhalation of contaminated particulate matter, as in smoking and occupational exposures, or by ingestion of contaminated food and water, as in environmental exposures. Animal data indicate a high absorption of Cd via the pulmonary route. Depending on the size and solubility of the Cd-laden particles, between 10 and 40 percent of the inhaled dose will be absorbed from the lungs (Friberg et al., 1974; 1977).

In contrast, only a small proportion of dietary Cd is absorbed from the gastrointestinal (G.I.) tract. The percentage of the dose absorbed appears to vary widely depending on the species of animals, the type of Cd compound used, the size and number of doses, and the technique employed to measure absorption (Bremner, 1979). Estimates of absorption ranged from 0.5 to 3 percent in rats, mice and monkeys to upwards of 7 percent in humans (Friberg et al., 1977). In reviewing the findings from several animal studies and from one study in which five human volunteers were given a single oral dose of radioactive Cd^{+2}, Friberg et al (1974) concluded that the average absorption of ingested Cd was 2 percent in animals and 6 percent in humans. Moreover, a poor nutritional status, characterized by low intake of calcium, iron and/or protein will increase the absorption to 10 percent or more (Friberg et al., 1974; 1977; Webb, 1975; Washko and Cousins, 1977).

After absorption from the lungs or the intestines, Cd is transported via the blood to the liver, kidney, and other tissues. With chronic exposure in animals, approximately 75 percent of the absorbed dose is accumulated in the liver and kidneys. In normal humans, after chronic, low-level, environmental exposure, about 50 percent of the Cd body burden is in the liver and kidneys combined and about one-third is in the kidneys alone (Friberg et al., 1974; 1977). The renal Cd burden is accumulated to a greater extent in the cortex than in the medulla. Thus, it is estimated that the concentration of Cd in the cortex is about 1.5 times the concentration in the whole kidney (Friberg et al., 1974; Kawai et al., 1976).

Recent Japanese studies (Kubota et al., 1974; Kyohno and Kawai, 1975; Nishizumi, 1975; Murakami et al., 1975) using both x-ray microanalysis and various staining techniques of thin tissue sections have demonstrated the intratissue distribution of Cd in chronically exposed dogs, rats and mice. Renal Cd localizes in the cortical proximal tubular epithelial cells, in the mesagium of the glomerulus, and in the walls of the renal arterioles.

The liver has a capacity to accumulate large amounts of Cd. During the early phases of exposure, the concentration of Cd in the kidney is often much less than that in the liver. However, with time, there is a slow transfer of Cd from the liver to the kidneys such that the ratio of liver:kidney Cd concentration gradually decreases (Bremner, 1979; Kjellstrom and Nordberg, 1978).



(From Kjellstrom and Nordberg, 1978).

(A) represents pulmonary exposure.
(G) represents gastrointestinal exposure.

Blood compartment B2 represents the accumulation of Cd^{+2} in the erythrocytes. This Cd is unavailable for distribution in the same manner as Cd bound to plasma proteins.

Blood compartments B1 and B3 are assumed to have a rapid Cd turnover and, therefore, no accumulation. Compartment B3 represents Cd bound to metallothionein, while B1 represents Cd bound to plasma proteins other than metallothionein, such as albumin.

It is assumed that all of the Cd from B3 is filtered through the renal glomeruli and a certain fraction is reabsorbed in the tubules to contribute to the renal Cd burden, while the remainder is excreted in the urine.

Figure 2-3. Flow Scheme of a Kinetic Model of Cadmium Metabolism

Upon administration of varying doses of Cd to experimental animals, the larger the dose, the larger the proportion of the dose that accumulates in the liver (Kjellstrom and Nordberg, 1978). The point at which the concentration of renal Cd exceeds that of hepatic Cd may be dose dependent. Perry et al. (1977) gave female Long-Evans rats 0.0, 1.0, 2.5, 5.0, 10.0, 25.0 and 50.0 μg Cd^{+2}/ml of drinking water for up to 30 months. The concentration of renal Cd exceeded the concentration of hepatic Cd within six months (i.e., at their first sacrifice point) for all but the 50 μg/ml group and within 12 months for this high dose group. Female Sprague- Dawley rats receiving $CdCl_2$ in the drinking water at low exposures of 0, 2 and 20 μg Cd^{+2}/ml for 12 months and a high exposure of 200 μg/ml for 8 months showed a similar response (Bernard et al., 1979). At the low exposures, the renal Cd concentration was greater than the hepatic Cd concentration for the entire exposure period; however, at the high exposure, the concentration of metal was equivalent in both organs. Human data are in accordance with these observations. Gross et al. (1976) measured the liver and kidney Cd concentrations in autopsy specimens from 107 people exposed to background levels of Cd. The ages ranged from a 19 week fetus to an 85 year old adult, and the concentration of Cd in the kidney exceeded that in the liver in all cases.

After absorption, Cd is transported to the various tissues via the blood. The metal is rapidly cleared from the blood in two phases. In experimental animals injected with Cd, most of the dose is found in the plasma immediately after exposure. With passing time after a single dose, or after repeated administration, most of the metal in the blood moves from the plasma and accumulates in the red blood cells (RBC). After repeated injections of Cd, blood concentrations of the metal tend to plateau, with most of the Cd being in the RBCs bound to the low molecular weight protein metallotionein and perhaps also to hemoglobin (Bremner, 1979; Webb, 1975; Friberg et al., 1977). It has been proposed that metallothionein functions as a Cd carrier in the blood and is involved in the transport of the metal from the liver to the kidneys (Bremner, 1979; Friberg et al., 1977).

Metallothioneins are a group of low molecular weight (6,000 to 7,000 m.w.) cytoplasmic metalloproteins having a high affinity for Cd, Hg, Zn, Ag and Cu. Although present in only trace amounts in normal tissue, metallothionein production is induced by exposure of the individual to sublethal doses of Cd or any of these other metals. When animals are treated with appropriate doses of Cd or certain other metals, synthesis of this protein takes place in the liver, kidney and spleen. High concentrations of Cu also induce synthesis of copperthionein in the liver and kidney. High concentrations of Zn (about 30 μg/g tissue) induce metallothionein synthesis in the liver, but not in the kidney. It is suggested that this may partly explain why Zn does not accumulate in the kidney to the extent that other heavy metals do during chronic exposures (Cherian and Goyer, 1978; Cousins, 1979).

In Cd-exposed animals, large quantities of metallothionein have been isolated from the liver and intestinal mucosa (Cherian, 1979; Cousins, 1979). Also, in organs such as kidney, liver, pancreas, and testicles, Cd is found to a great extent bound to metallothionein. It is suggested that transport of Cd from the gut is mediated by a Cd-metallothionein complex (Friberg et al., 1977). Metallothionein acts as an intracellular storage protein which firmly binds Cd, thereby detoxifying the metal by making it unavailable for interaction with essential enzymes and functional processes. The fact that prolonged low-level exposure of experimental animals to ingested or injected Cd can result in the accumulation of high Cd^{+2} body burdens without significant adverse effects may be attributable to the detoxification properties of metallothionein (Webb, 1979; Cherian and Goyer, 1978).

Using an indirect immunoperoxidase staining technique on fixed sections of rat liver, kidney, small intestine, lung and testis, Danielson et al. (1982, 1982a) found that metallothionein synthesis and accumulation is tissue and cell-type specific. In the kidney tissues from both control and Cd-treated animals, this protein was confined intracellularly to epithelial cells of the renal tubules. In the cortex, many of the proximal tubular cells contained metallothionein and material within the tubular lumina frequently stained positive. In the medulla, the tubular cells of the collecting ducts stained strongly for this protein. In contrast, metallothionein was not detected in squamous epithelial cells of the glomeruli, Bowman's capsule, blood vessels or interstitial connective tissue. The authors suggested that, in addition to its commonly believed role in detoxification of heavy metals, metallothionein may be involved in metal storage or transport.

There appears to be a controversy over the theorized role of a Cd-thionein complex as the vehicle for transport of the metal from the liver to the kidney. Friberg et al. (1974; 1977) pointed out that the low molecular weight of metallothionein allows this protein to be filtered through the glomerular membrane and subsequently be reabsorbed into the renal tubules. Thus, such a mechanism could explain the selective accumulation of Cd in the renal cortex. In contrast, Cherian and Goyer (1978) noted that most experimental evidence does not support this idea. These authors cited the potent nephrotoxic effects of Cd-thionein. Apparently, even trace amounts of Cd-thionein in the plasma cause renal damage (Cherian, 1979). It is argued further that in chronic Cd exposure, the toxic symptoms, especially the nephrotoxic effects, appear only after a significant lag period. Therefore, if Cd was transported to the kidney as Cd-thionein, the nephrotoxic effects would be expected to appear much earlier. Cherian and Goyer (1978) suggested that Cd is transported from the liver to the kidney via chelation by small molecular weight sulfur-containing amino acids or by weak binding to plasma proteins such as albumin.

Cherian (1979) gave six to ten week old male mice oral doses of radiolabelled $^{109}CdCl_2$ and ^{109}Cd-thionein and observed the organ distribution of these materials four hours after dosing. The results showed that Cd-thionein reached the small intestine intact and probably was absorbed without much degradation. In contrast, a major portion of the renal Cd was bound to high molecular weight proteins, and not to metallothionein. Since only trace amounts of the labeled Cd-thionein could be found in the kidney, it was concluded that ingested Cd-thionein, although absorbed intact from the gut, was degraded considerably during transfer to the kidney or soon after deposition in the kidney.

Cousins (1979) has proposed that as Cd enters the intestinal mucosal cell, it helps induce the production of intestinal metallothionein. The protein sequesters a major part of the dietary Cd that is taken up by the mucosal cells.

Cd that is transported via the plasma is first taken up by the liver cells and incorporated into hepatic metallothionein. In the liver, Cd-thionein undergoes degradation to release Cd which then binds to newly synthesized metallothionein or interacts with other cellular components. Later, Cd appears to be deposited in the kidney, bound primarily to metallothionein which is synthesized in the renal cells.

Figure 2-3 reflects the dynamic interaction of the blood and the liver, kidneys and other tissues involved in distributing and accumulating the Cd to which the body is exposed. As indicated, the major routes of excretion are via the feces and urine. Human and animal data both show that only a very small part of the absorbed Cd is actually excreted. Thus, in humans, only about 0.005 to 0.01 percent of the total Cd body burden is excreted (Friberg et al., 1977).

Fecal Cd includes both the nonabsorbed part of ingested Cd and the "true" fecal excretion. As noted previously, absorption of dietary Cd is very low, ranging from about two to six percent in experimental animals and man, respectively. Thus, about 95 to 98 percent of the ingested Cd is promptly excreted in the feces (Bremner, 1979; Friberg et al., 1974). The "true" fecal excretion reflects the portion of the Cd body burden that is eliminated. This part of the fecal excretion includes contributions from pancreatic and bile secretions plus Cd contained in desquamated epithelial cells of the gastrointestinal mucosa (Bremner, 1979; Friberg et al., 1974; Kjellstrom and Nordberg, 1978).

Excretion of Cd via the urine is generally small and insignificant in terms of decreasing the body burden. In the absence of overt renal damage, the excretion via the urine increases with age (on a group basis) and is proportional to the body burden of Cd (Friberg et al., 1974; 1977). With the onset of renal tubular damage, the excretion of urinary Cd increases dramatically in concurrence with the onset proteinuria.

Suzuki (1981), giving rats daily injections of 0.5 mg Cd^{+2}/kg for up to 25 weeks, found that Cd exposure also increased the urinary excretion of copper and zinc. Prior to renal damage, metallothionein was directly involved in the excretion of copper. With the onset of proteinuria and renal tubular dysfunction, the Cd excretion greatly increased and metallothionein was associated with the elimination of both Cd and copper.

The long biological half-life of Cd, ranging from about 200 days in the rat to upwards of 33 years in the human kidney, results from this extremely slow elimination via urine and feces (Webb, 1975).

RENAL EFFECTS FROM CADMIUM EXPOSURE

During chronic exposure to Cd, the target organ is the kidney. In both man and experimental animals, chronic exposure can result in renal tubular dysfunction which manifests as proteinuria (elevated urinary protein), glycosuria (elevated urinary glucose), amino aciduria (elevated urinary amino acids), polyuria (elevated urine volume) and enzymuria (elevated urinary enzymes) (Friberg et al., 1974; Chang et al., 1981; Samarawickrama, 1979).

Based on available animal and human data, Friberg et al. (1974) have suggested that a concentration of 200 μg Cd/g wet weight of kidney cortex is the critical concentration that can result in detectable renal dysfunction. This value represents an average sensitivity to Cd; however, due to individual variations in sensitivity, renal dysfunction may occur at cortical concentrations lower than 200 μg/g. Thus, some animal data indicate renal effects occurring at significantly lower concentrations. Male SD-JCL rats were given 10, 50, 100, and 200 μg Cd/ml in the drinking water for 37 weeks (Kawai et al., 1976). Renal tubular atrophy and interstitial edema were found in animals having a renal Cd^{+2} concentration as low as 44.4 μg Cd/g wet weight of tissue. Female Wistar rats exposed to $CdCl_2$ in the drinking water for 90 days showed significant proteinuria, compared to controls, at average levels of 27.22 and 36.38 μg Cd/g wet weight of kidney (Prigge, 1978).

Many animal studies (Kawamura, et al., 1978; Kajikawa et al., 1981; Itokawa, 1974; Kawai, et al., 1976) have shown that chronic Cd intake is associated with renal morphological changes that are largely confined to the cortical proximal tubules. At the histological level, these changes include: 1) proximal tubule cell damage ranging from mild swelling to atrophy, degeneration, or necrosis of epithelial cells, and 2) interstitial cell damage ranging from slight edema to fibrosis or interstitial nephrosclerosis.

Kajikawa et al. (1981), studying the renal effects on male Wistar rats exposed to 200 μg/ml $CdCl_2$ in the drinking water for up to 91 weeks, found that even after long-term exposure, Cd did not affect all parts of the tubules or tubular cells to an equal extent. Thus, histological changes in each kidney varied considerably from tubule to tubule and even from individual cell to cell within a single tubule. In their work, the earliest detectable changes in the proximal tubules involved swelling of the tubular epithelial cells, followed later by enlargement and occasional hypertrophy of the nuclei. Similar nuclear changes, although less pronounced, were found in the control rats. As the exposure progressed, more tubular cells were found to be necrotic, with cytoplasmic eosinophilia and pyknotic nuclei, while other cells exhibited loss of brush border and extrusion into the tubular lumina.

Some studies involving rats and rabbits (Itokawa, et al., 1974; Nomiyama, et al., 1973; Kawai et al., 1976) have shown that Cd also can induce degeneration of renal glomeruli. Such lesions included necrosis and partial hyalinization of glomerular capillaries, and adhesions between Bowman's capsule and glomerular tufts.

In contrast, Kajikawa et al. (1981) found no remarkable alterations in the glomeruli or tubular segments other than the proximal tubules.

It has been pointed out (Kajikawa et al., 1981; Kawai et al., 1976) that the evaluation of Cd-induced renal pathology during chronic exposure studies is complicated by spontaneous nephropathy, which occurs naturally in elderly animals, particularly in certain strains of rats. Kajikawa et al. (1981) noted that spontaneous nephropathy is easily distinguishable as it involves multiple hyaline casts in the convoluted tubules and collecting ducts, atrophy of the glomeruli plus fibrosis and lymphocytic infiltration around the affected glomeruli and tubules. Kawai et al. (1976) cautioned that once this age-related disease has occurred, it may obscure the lesions due to Cd exposure.

Of particular interest in assessing the relationship between normal renal aging and Cd-induced renal pathology, is the finding of Kajikawa et al. (1981) that the incidence of spontaneous nephropathy throughout their study was higher in the Cd-exposed rats (56.7 percent) than in the controls (16.0 percent). Because this disease is age-dependent, its incidence increased in both groups such that after 52 weeks, all of the remaining experimental rats and 33.9 percent of the control rats were affected. Based on these findings, it was suggested that exposure to Cd may promote age-related renal changes in the rat.

Electron microscopic studies (Nishizumi, 1975; Kajikawa et al., 1981; Squibb et al., 1979) have been conducted to determine the ultrastructural changes induced in the kidney by Cd exposure. Such changes have been found almost exclusively in the epithelial cells of the proximal convoluted tubules. These alterations include mitochondrial swelling, proliferation of smooth endoplasmic reticulum (SER), increased number of lysosomes and nuclear inclusion bodies, coagulative necrosis and destruction of the brush border. Kajikawa et al. (1981) found that even after 91 weeks of dosing rats with 200 µg Cd^{+2}/ml in the drinking water, there was variable susceptibility among the tubular cells to such ultrastructural changes. Within any kidney, there were mixed populations of relatively intact tubular cells, mildly affected cells having increased pinocytic vesicles and SER, and severely injured cells with vacuoles and focal necrosis.

Kajikawa et al. (1981) found an increased number of apical pinocytic vesicles in the proximal tubular epithelium of Cd-dosed rats and interpreted this as representing active reabsorption of the Cd-thionein complex that transports Cd from the liver to the kidneys. Previous work (Squibb et al., 1979) to elucidate the mechanism of Cd-induced cellular damage supports this interpretation.

Squibb et al. (1979) administered intravenous ^{109}Cd-thionein to male Sprague-Dawley rats at doses of 0.017 and 0.17 mg Cd^{+2}/kg and observed renal uptake of $^{109}Cd^{+2}$ and ultrastructural changes in the proximal tubular cells for up to 24 hours after dosing. Circulating Cd-thionein was reabsorbed by the proximal tubules by the same mechanisms involved in reabsorption of other low molecular weight plasma proteins. The data suggest that Cd-thionein is reabsorbed into the proximal tubule cells via pinocytosis and is degraded by lysosomes following the fusion of the pinocytotic vesicles with primary lysosomal bodies. Cd released in or from the lysosomes following the degradation step may be responsible for the development of tubular necrosis. The authors speculated that Cd remaining within the lysosomes might inhibit normal lysosomal function and thus contribute to cell death by preventing the cell from degrading other reabsorbed proteins, such as albumin, and thereby upset the intracellular osmotic conditions.

Similarly, other investigators (Fowler and Nordberg, 1979) attributed cell death to the liberation of toxic Cd from lysosomes in excess of the synthesized metallothionein needed to resequester the ions. As pointed out by Friberg et al. (1974), once the renal metallothionein is saturated, freed Cd will exchange with Zn in Zn-dependent enzymes, such as leucine aminopeptidase, that are necessary for reabsorption and catabolism of proteins by the proximal tubule cells.

Also of interest as a possible mechanism for explaining the cellular effects of Cd exposure, Kajikawa et al. (1981) found that vacuolar degeneration of the tubular cells was a pronounced feature of late stage Cd exposure in rats. This effect apparently developed as a consequence of increased size and number of pinocytic vesicles and lysosomes. Since the evolution of vacuolar degeneration correlated with increased excretion of urinary proteins, it was concluded that this type of ultrastructural change may be closely related to the impairment of tubular reabsorption.

Based on current animal data, the pathophysiological changes that occur in the kidney as a result of Cd exposure have been summarized (Friberg et al., 1974; Chang et al., 1981) as follows:

1. Production of metallothionein, mainly in the liver, is induced by Cd, which then binds with the produced metallothionein. The Cd-metallothionein complex is reabsorbed by the renal proximal tubules and is deposited in the tubular epithelial cells, thereby causing a marked increase in the concentration of Cd in the renal cortex.

2. With increasing exposure, more CD accumulates in the kidney than can be bound to metallothionein and the excess exchanges with Zn in Zn-dependent enzymes, such as leucine aminopeptidase. In so doing, Cd has an inhibitory effect on these enzymes, which normally function in the protein reabsorption and catabolic activities of the proximal tubules. Such disturbance of normal enzyme and tubular functions results in proteinuria and aminoaciduria. Concurrently, there is a decrease in the tubular reabsorption of the Cd-metallothionein protein complex, which leads to increased Cd excretion in the urine and a decreased rate of Cd accumulation in the kidney.

3. The renal tubular cells eventually are injured by the Cd and undergo pathological changes. Damaged epithelial cells are desquamated into the tubular lumen and are excreted in the urine. Since Cd is excreted along with these cells, there is a corresponding marked decrease of renal Cd levels plus an increase in urinary Cd and protein. After prolonged exposure to Cd, resulting glomerular damage causes further increases in proteinuria.

EFFECT OF CADMIUM ON THE ACCUMULATION AND METABOLISM OF COPPER AND ZINC

Cd can affect enzymes and processes which require Zn, Cu and Fe for their stability, integrity and/or function. Also, iron and copper metabolism are closely related so that the effect of Cd on one may easily involve interaction with the other. Existing animal data indicate that ingested Cd^{+2}, even at relatively low doses, can markedly alter zinc, copper, and iron metabolism even when the diets are adequately fortified with these essential metals

(Petering et al., 1979). There are complex antagonistic interactions of Cd, Zn, Fe and Cu in animals. Elevated dietary Cd can produce deficiency states of these essential metals resulting in various physical problems such as reduced growth and anemia (Bremner, 1979).

It has been suggested that Cd influences the metabolism of Cu and Zn because of the similar chemical properties of these metals. Also, the likely involvement of metallothionein in these interactions cannot be overlooked (Bremner, 1979). Webb (1975) noted that the mechanism for the antagonism of Cu and Zn metabolism by Cd was unknown; however, it may involve competition for transport sites. In support of this idea is the fact that the accumulation of Cd-thionein in Cd-exposed animals results in excessive concentrations of hepatic Zn and renal Cu.

El-Gazzar et al. (1979) gave female Sprague-Dawley rats 0.0, 4.3, 8.6 and 34.4 µg Cd^{+2}/ml in the drinking water and examined subcellular distribution of Cd and Zn in the liver after 200 days. Although the number of animals examined was very small (total of seven), the data showed a positive correlation between increasing Cd dose and increasing Cd and Zn in the liver. Animals in the two higher doses synthesized hepatic metallothionein containing both Cd and Zn.

The same effect was seen in female Long-Evans rats given up to 50 µg Cd^{+2}/ml in the drinking water for up to 24 months (Perry et al., 1977). Animals were sacrificed every six months. At each sacrifice, there was a significant positive correlation between increasing dose in the water and increasing hepatic Zn and Cd concentrations. In contrast, at each dosage level, there was only a slight increase of hepatic Zn with increasing age.

Several studies also have shown that oral administration of Cd in the drinking water of rats alters the concentrations of copper and iron in the kidneys and serum. Renal levels of Cu and hepatic levels of Zn have been shown to vary directly with the concentration of Cd in the drinking water. In contrast, hepatic Cu levels showed no such variation (Petering et al., 1979).

Female Long-Evans rats exposed to none, low or moderate Cd in the drinking water for up to 24 months had renal Cu concentrations of approximately 10 µg/g wet weight. Rats with prolonged high exposure to Cd (50 µg/ml) showed an apparent increase of renal Cu up to about 40 µg/g wet weight (Perry et al., 1977).

Stonard and Webb (1976) examined the influence of dietary Cd on the distribution of renal and hepatic Zn, Cu and Fe by feeding male and female Wistar rats a powdered diet containing 100 mg Cd^{+2}/kg of food for about 50 weeks. The renal accumulation of Cd continued throughout the experiment and did not reach a plateau. Synthesis of renal and hepatic Cd-thionein was induced by exposure to Cd and throughout the experiment, 85 to 90 percent of the total Cd in either organ was recovered as the metallothionein. The progressive accumulation of Cd was accompanied by an increase in the concentration of Zn in the liver and of Cu in the kidney, both of which correlated with the synthesis of Cd-induced metallothionein. The most significant effect was seen in the increased concentration of renal Cu in the males. In both organs, excess Zn was stored as Zn-thionein; however, in response to Cd exposure, there was little increase of renal Zn-thionein above control levels. In contrast, renal thionein that was synthesized in response to Cd uptake also accumulated significant amounts of Cu, such that all the excess renal Cu was stored as Cu-thionein. It should be noted that there was a large age-related increase in the renal Cu concentration in the controls, ranging from a mean of 6.4 μg/g (wet weight) at 5 days to 21.3 μg/g at 36 weeks. In the dosed rats, mean Cu^{+2} concentrations at comparable times were 5.8 μg/g and 47.1 μg/g, respectively. Finally, Cd accumulated to low concentrations in the spleen, pancreas and heart, but did not induce measurable synthesis of metallothionein and did not affect the concentrations of Zn or Cu in these organs.

Piscator and Lind (1972) found somewhat different effects in the renal cortex of normal humans exposed to environmental Cd. Examining the metal concentrations of the kidney cortex from 67 Swedish people aged six to 91 years, these investigators found that up to age 50 years, there was a positive correlation between increasing Cd and Zn concentrations and increasing age. Thereafter, the concentrations of Cd and Zn both decreased with increasing age. It was noted that in most other organs, the Zn levels have been found to be fairly stable throughout life; however, in these kidneys, the accumulation pattern of Zn paralleled that of Cd. In contrast, because the levels of renal Cu did not vary with age, it was surmized that Cu metabolism in the kidney will not be disturbed when there is no excessive exposure to Cd.

THE EFFECT OF AGE ON THE ACCUMULATION OF RENAL CADMIUM

Chronic Cd feeding studies in animals (Perry et al., 1977; Nomiyama, 1974; Kajikawa et al., 1981) have demonstrated that the renal Cd concentration correlates positively with age and increasing Cd dose in young and middle aged individuals; however, the metal concentration decreases in the old animal. The same effect is seen in renal autopsy tissue from people not exposed occupationally to Cd (Travis and Haddock, 1980; Gross et al., 1976; Piscator and Lind, 1972).

Perry et al. (1977) gave female weanling Long-Evans rats 0.0, 1.0, 2.5, 5.0, 10.0, 25.0 and 50.0 μg Cd^{+2}/ml in the drinking water for up to 30 months and determined the concentrations of renal Cd every six months. In the 10, 25 and 50 μg/ml groups, renal Cd increased steadily up to age 18 months (peak levels of 78.5, 162.0, 222.0 μg Cd/g wet weight kidney, respectively), and then decreased markedly thereafter. The 1.0, 2.5, and 5.0 μg/ml groups increased continuously up to age 24 months (peak levels of 12.3, 32.0, 50.4 μg Cd/g wet weight kidney, respectively), and then decreased gradually thereafter. The investigators considered that this decrease could have been artifactual since it was observed in the relatively few animals that survived to 30 months. However, in view of data from other studies, it seems reasonable that this decrease is age-related and not artifactual.

Nomiyama (1974) fed rabbits 300 μg Cd^{+2}/g in their food for up to 54 weeks and found that the concentration of Cd in the renal cortex reached a peak of 250-300 μg/g wet weight in the 20th to 30th week and then decreased significantly thereafter.

Kajikawa _et al_. (1981) administered 200 μg/ml $CdCl_2$ in the drinking water of male Wistar rats and found that the renal cortical Cd concentration increased to about 200 μg/g wet weight by age 40 weeks and then declined rapidly until termination of the study at 91 weeks.

In humans not exposed occupationally to Cd, the concentration of renal Cd also shows a definite age-dependent pattern. In general, the Cd concentration increases steadily from birth, peaks around age 50 years, and decreases thereafter. The mean Cd concentration in the kidney cortices of 67 Swedish people, aged six to 50 years, started at 33 μg/g dry weight (mean age 13 years); peaked at 140 μg/g (mean age 55 years) and declined to 72 μg/g (mean age 84 years) (Piscator and Lind, 1972).

Similar results were found in the autopsy tissue of 107 Americans. The mean renal Cd concentration was 0.07 μg/g wet weight (in fetuses), then increased to 39.4 μg/g (in adults age 45-54 years) and declined to 20.9 μg/g (in adults aged 65+ years) (Gross _et al_., 1976).

In order to construct a model to explain the time-dependent pattern of renal Cd accumulation in humans, Travis and Haddock (1980) considered several factors that could explain the observed decline in renal Cd concentration in older people. They looked at the following factors:

1. Functional and morphological changes in the kidney that may cause increased Cd elimination in older people.

2. Age-related exposure variations that could occur as a result of age-dependent changes in dietary habits, caloric intake and smoking patterns.

3. Cohort-related exposure variations that could result from the more recent increases in environmental levels of Cd.

4. A decreased survival of people having higher renal Cd concentrations.

After examining the relative importance of each of these factors, the authors concluded that increased renal elimination of Cd due to age-related changes in the structure and function of the kidney had the greatest effect and constituted the most plausible explanation for the observed renal Cd decline. They cited structural changes in the human kidney such as loss of nephrons with age, alterations in the renal arterial tree, thickening of the glomerular and tubular basement membranes and a general shrinkage of the kidney with age. Functional changes included increased blood pressure, loss of ability to concentrate urine and decreased tubular reabsorptive capacity.

On the basis of existing empirical and indirect evidence relating to both renal Cd kinetics and renal age-related changes, Travis and Haddock (1980) constructed a model which used an assumed time-dependent Cd excretion rate constant that reflected age-dependent functional and morphological renal changes. The excretion rate constant was assumed to be fixed until age 30 years and then gradually increased in a linear fashion with age. It was determined that the decline in renal Cd concentration observed in older age groups could be well explained by this age-dependent Cd excretion rate taken in conjunction with an age-dependent caloric intake, a change in food Cd concentration with time, and the effects of smoking.

CADMIUM-INDUCED CHANGES IN URINE PARAMETERS

Chronic Cd exposure in both man and laboratory animals can cause abnormalities of renal tubular function which result in low molecular weight (m.w.) proteinuria (elevated urine, low m.w. protein), glycosuria (elevated urine glucose), polyuria (elevated urine volume), aminoaciduria (elevated urine amino acids), and enzymuria (elevated urine enzymes) (Samarawickrama, 1979; Friberg et al., 1974).

Proteinuria

Proteinuria results not only from Cd-intoxication, but also from chronic renal disease associated with various conditions, including normal aging. Berg (1965), studying normal male Sprague-Dawley rats, found the mean daily output of urine protein to increase from less than 1 mg/24 hours at age one month to about 212 mg/24 hours by age 21 months. Electrophoretic examination of these proteins showed that the principal early proteins (up to age four months) were similar to serum α_2- and β-globulins. A protein similar to serum albumin appeared by age 12 months and then predominated with increasing age and increasing proteinuria.

Cd-induced proteinuria in humans is different from the proteinuria associated with acute and chronic nephritis or the nephrotic syndrome (Vigliani, 1969). Nephritis and nephrotic syndrome are renal diseases that impair glomerular and tubular function and result from various etiologies, including aging. Proteinuria arising from nephritis consists primarily of high m.w. proteins - albumin and globulins in the urine (Koushanpour, 1976). In most diseases associated with significant proteinuria, albumin is the major component of the urine proteins (Kurtzman and Rogers, 1974).

In contrast, Cd-induced proteinuria is characterized by the presence of low m.w. proteins in the urine. This is a tubular type proteinuria that results from a decreased reabsorption in the proximal tubules of serum proteins present in the glomerular filtrate. Gel filtration, ion exchange chromatography, and immunological methods have been used to characterize the protein components in urine from Cd-exposed workers. There is a predominance of alpha-, beta-, and gamma-globulins. A large number of low m.w. (m.w. below 40,000) serum proteins have been identified, including β_2-microglobulin, ribonuclease, muramidase, lysozyme, immunoglobulin light chains, and retinol-binding protein (RBP). An increase in low m.w. urine proteins, particularly β_2-microglobulin (β_2-m) is considered a first sign of Cd-induced renal dysfunction. Further, an increase of β_2-m may be present without a concomitant increase in total protein excretion or any major changes in the electrophoretic pattern of the urine proteins (Piscator and Pettersson, 1977; Nomiyama, 1981; Kjellstrom and Piscator, 1977; Samarawickrama, 1979). Thus, the radioimmunoassay of β_2-m in the urine is a sensitive indicator of Cd-induced proteinuria and may be one of the most sensitive methods for detecting early tubular damage (Shiroishi et al., 1977; Kjellstrom and Piscator, 1977).

Nogawa et al. (1978), studying the dose-response relationship between environmental Cd exposure (in Japanese people eating heavily contaminated rice) and renal tubular dysfunction, found that tubular proteinuria was not associated with normal aging. Rather, the prevalence of tubular proteinuria and renal dysfunction correlated with increasing age only when there was a concomitant increase in Cd exposure.

Peterson et al. (1969) measured the β_2-m, albumin, and total protein content of 24-hour urine samples from healthy people, and individuals with proteinuria of either tubular or glomerular origin. Three people with chronic cadmium poisoning were included with the tubular proteinuria group. In healthy subjects, the average 24-hour urinary excretion was 0.12 mg β_2-m, 10 mg albumin and 80 mg total protein. Patients with renal glomerular disorders had normal to slightly elevated β_2-m, but significantly elevated albumin and total protein. Chromatograms of these proteins showed a predominance of proteins of relatively large molecular size, with albumin forming the largest peak. Those with tubular dysfunctions excreted large amounts of β_2-m, but only normal or slightly elevated albumin and only moderately increased total protein. Chromatograms of these tubular proteins were characterized by relatively large amounts of small molecular size proteins, eluted late in the chromatogram and having a distinct peak at the β_2-m position. The ratio of urinary albumin: β_2-m was high (1,100 to 14,200) in glomerular proteinuria, intermediate (33 to 163) in normal proteinuria and low (1.0 to 13.3) in tubular proteinuria.

Many epidemiological studies have been conducted to investigate the relationship between Cd exposure, renal pathology, and diagnostic urine parameters, including total protein and β_2-m. Studies on environmental exposures resulting from chronic ingestion of Cd-contaminated rice (Shiroishi et al., 1977; Nogawa et al., 1978; Kojima et al., 1977; Kjellstrom et al., 1977) and studies on occupational exposures resulting from inhalation of Cd containing dusts, fumes, or aerosol (Kjellstrom et al., 1977; Bernard et al., 1979; Adams et al., 1969; Sandifer et al., 1979) have demonstrated the dose-response relationship between exposure duration or employment time and the occurrence of tubular proteinuria and dysfunction.

More recently (Bernard et al., 1979), it has been shown that after prolonged occupational exposure to Cd, the resulting proteinuria has two components: 1) a tubular proteinuria characterized by elevated excretion of β_2-m, and 2) a glomerular proteinuria characterized by elevated excretion of high molecular weight proteins such as orosomucoid (m.w. 44,000), albumin (m.w. 69,000), transferrin (m.w. 76,500) and IgG (m.w. 160,000). Although each component can appear independently, they often are associated together, giving rise to a mixed type of proteinuria. In this study, such proteinuria was observed primarily in workers exposed to Cd for more than 25 years and whose Cd concentration in the blood exceeded 1 μg Cd/100 ml and that in the urine 10 μg Cd/g creatinine.

The clinical significance of a slight increase in β_2-m apparently is not yet known. When Cd-induced tubular damage has progressed to a state of high urinary β_2-m as well as increased total protein, then the tubular damage is irreversible and will persist even after cessation of Cd exposure (Friberg et al., 1974; Kjellstrom and Piscator, 1977; Shiroishi et al., 1977).

Using various types of lab animals, several investigators have demonstrated a causal relationship between Cd exposure and proteinuria. It has been suggested that a renal Cd concentration of about 200 μg/g wet weight in the cortex is the critical level at which morphological and functional changes, such as proteinuria, appear (Friberg et al., 1974). However, due to individual variation in sensitivity, renal dysfunction may start at values higher or lower than this (Samarawickrama, 1979).

Rabbits injected subcutaneously (s.c.) with 0.25 mg $CdCl_2$/kg/day for five days per week developed proteinuria after one month. Early proteinuria consisted of increased excretion of immunoglobulin light chains and correlated with some cloudy swelling of the proximal convoluted tubular cells. Later, the proteins consisted of light chains, albumin, alpha-, beta-, and gamma-globulins and there was gross tubular damage (Vigliani, 1969).

Kajikawa et al. (1981) found that in male Wistar rats given 200 μg/ml $CdCl_2$ in the drinking water for up to 91 weeks, progressive changes in the proximal tubules coincided with increased renal cadmium levels and increased urinary excretion of total protein. In female Wistar rats given 50 and 100 μg/ml $CdCl_2$ in the drinking water for 90 days, Prigge (1978) found significant increases in proteinuria, corresponding to mean cortical Cd concentrations of 27.22 and 36.38 μg/g wet weight, respectively. Proteinuria was not seen in control rats or rats given 25 μg/ml $CdCl_2$. The 25 μg/ml group had a mean renal cortical Cd concentration of 13.11 μg/g wet weight.

Axelsson and Piscator (1966) exposed rabbits for 11, 17, 23 and 29 weeks to 0.25 mg/kg of $CdCl_2$ given as s.c. injections five times a week. After 18 weeks of exposure, there was a significantly greater excretion of protein in the exposed animals, as compared to the controls. This coincided with the peak Cd concentration in the renal cortex which occurred at 17 weeks and had a mean value of roughly 400 μg/g wet weight. Urine proteins from control and exposed rabbits were examined by paper and starch gel electrophoresis. The exposed animals had a low albumin content, but a fairly large alpha-, beta-, and gamma-globulin fraction. In the controls, the amount of protein and its electrophoretic pattern showed considerable interindividual variation. Some controls had albuminuria and transient increases in the alpha-fraction.

In reviewing cadmium-related studies conducted in Japan, Tsuchiya (1978) summarized the findings from a few chronic rat and mouse studies in which Cd was administered in the drinking water. It was noted that although proteinuria occurred in many of the test groups, its prevalence generally was not significantly different from that in the controls. It is well known that during long-term experiments, normal mice and rats often develop spontaneous nephritis, with concomitant proteinuria. Thus, proteinuria should be evaluated with caution in chronic cadmium studies.

More recently (Katagiri et al., 1973) a study using rabbits indicated the possibility that Cd also may damage the glomeruli. Five rabbits were given daily s.c. injections of 2 mg/kg $CdCl_2$ for 24 days. Total protein gradually increased up to day 14 and consisted primarily of low molecular weight (m.w. 10,000 to 20,000) proteins. Thereafter, the total protein increased sharply; however, there was a greater proportion of high molecular weight proteins than low molecular weight proteins. The authors concluded that Cd-induced renal dysfunction develops first in the renal tubules and then at a later stage extends to the glomeruli.

Glycosuria

The presence of glycosuria is dependent on three factors: 1) the concentration of glucose in the blood, 2) the glomerular filtration rate (GFR), and 3) the degree of glucose reabsorption in the proximal tubules. Normal urine is glucose-free; however, when the filtered load of glucose exceeds the reabsorptive capacity of the tubules, glucose will appear in the urine. Glycosuria of renal origin, unlike that associated with diabetes mellitus, results from reduced tubular reabsorption of glucose in the presence of normal blood glucose levels. Glycosuria of renal origin occurs in people with Fanconi syndrome, Wilson's disease, and as a result of exposure to certain nephrotoxic agents such as cadmium (Kurtzman and Rogers, 1974; Koushanpour, 1976).

Several epidemiological studies investigating Cd-related health effects have demonstrated a causal relationship between Cd exposure and glycosuria in people exposed both environmentally (i.e., through ingestion of contaminated water and food) and occupationally.

Shiroishi et al. (1977) examined the urines from Itai-itai patients, Itai-itai observation patients (i.e., those with kidney but no bone symptoms), glomerulonephritis patients, and normal controls. The Itai-itai and Itai-itai observation patients had been exposed for many years to high levels of Cd in the food and water and a high prevalence of glycosuria. All the Itai-itai and 25 of the 32 Itai-itai observation patients had glycosuria, as measured qualitatively by a test tape reaction. This correlated with a positive tubular type proteinuria in all these patients. In contrast, none of the glomerulonephritis patients and only two of the 17 controls had glycosuria. This finding corresponded with 14 of the 17 controls having a normal proteinuria and 7 of the 9 glomerulonephritis patients having a glomerular type of proteinuria.

Nogawa et al. (1978), studying the dose-response relationship between environmental Cd exposure (i.e., through ingestion of contaminated rice and water) and renal dysfunction, found a positive correlation between the prevalence of glycosuria and increased urine excretion of Cd (expressed as µg/g creatinine). Similarly, Kjellstrom et al. (1977) reported that pathological urinary findings, including glycosuria, were strongly related to Cd exposure time. In this study, exposure of Japanese farming women was estimated from the individual's length of residence in a Cd-contaminated area and the Cd concentration of the rice grown and eaten in that area.

In cadmium workers, the appearance of a mild, yet persistent proteinuria is considered to be the earliest renal effect. Depending on the extent of Cd exposure, there may be a disturbance in the renal handling of other filtered components, such as glucose, so that proteinuria becomes only one aspect of a more generalized renal tubular dysfunction (Kazantzis, 1979). Since glycosuria is not a consistent finding in occupationally exposed populations, some consider this condition not to be an early sign of Cd-induced renal effects (Nomiyama, 1981).

Studying renal dysfunction in men exposed to cadmium oxide dust in an alkaline accumulator factory, Adams et al (1969) examined two groups of workers: 1) 19 men with variable lengths of employment and all having diagnosed proteinuria, and 2) 10 men exposed to Cd for at least 10 years but not having proteinuria. The mean urinary Cd concentration was 94 µg/l for those with proteinuria and 28 µg/l for those without proteinuria. Seventy-eight percent of the proteinuric men and only ten percent of the non-proteinuric men had urinary glucose concentrations exceeding 15 mg/100 ml. The range of glucose values was 2 to 700 mg/100 ml for the proteinuric men and 1.8 to 160 mg/100 ml for the non-proteinuric men.

Kazantzis et al. (1963) found glycosuria and tubular proteinuria in two out of 12 workers exposed to cadmium sulfide dust for 25 to 30 years. Three other men with equivalent exposure times had tubular proteinuria but no glycosuria. Four men with 12 to 14 years of exposure had no glycosuria but three of them had tubular proteinuria. Three men with less than two years of exposure had neither glycosuria nor proteinuria. Sixteen years later, a follow-up study (Kazantzis, 1979) was conducted on six men with the longest exposures. All six still had tubular proteinuria, five also had glycosuria, and each had several other urine and blood abnormalities consistent with Cd-induced pathology.

Piscator and Pettersson (1977) indicated that a slight glycosuria often might be present but would not be detected by the test papers commonly used in such studies. By using a glucose-oxidase reagent for the quantitative determination of urinary glucose, it was found (Piscator, 1962) that the urinary excretion of total protein did not correlate with the excretion of glucose in Cd-exposed workers. Thus, in some cases with high total protein, the glucose excretion was normal. In general, however, it was found that workers excreting 200 to 1,300 mg protein/day also excreted 100 to 500 mg glucose/day.

In Cd-exposed animals, glycosuria has been found to appear concurrently with or shortly after the onset of proteinuria. Axelsson and Piscator (1966) reported concurrent glycosuria and proteinuria after 23 days in five rabbits given s.c. injections of $CdCl_2$ five times/week at a dose of 0.25 mg Cd^{+2}/kg. They also found a significant negative correlation between glucose reabsorption and protein excretion. Thus, increasing levels of glucose were excreted with increasing amounts of protein.

Nomiyama et al. (1975) fed rabbits pelleted food containing 300 ppm Cd^{+2} as $CdCl_2$ and observed changes in renal function for 54 weeks. The earliest signs of intoxication were aminoaciduria and enzymuria, with both detected after 14 to 16 weeks. The prevalence of proteinuria was significantly higher in the dosed animals, as compared to the control, after 38 weeks. The prevalence of glycosuria was significantly higher after 42 weeks. The prevalence of glycosuria was much lower than that of proteinuria, and glycosuria appeared independent of proteinuria in some rabbits. Since rabbits with glycosuria survived as long as rabbits without glycosuria, the authors felt that this parameter could not be used as a sign of severe Cd intoxication.

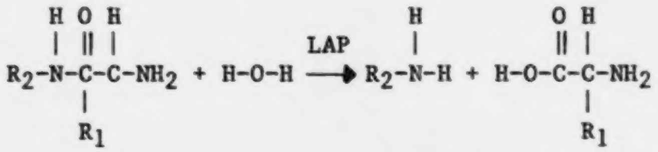
Rabbits given s.c. injections of $CdCl_2$ at dose levels of 1.5, 6.0 and 15.0 mg Cd^{+2}/kg/day showed early signs of proteinuria. Glycosuria appeared after the onset of proteinuria. The length of time between onset of proteinuria and onset of glycosuria varied inversely with the magnitude of the Cd dose (Nomiyama et al., 1973).

Yoshikawa (1967) gave mice s.c. injections of either $CdCl_2$ or cadmium stearate for 30 days at a dose of 0.5 mg Cd^{+2}/kg. In mice given $CdCl_2$, proteinuria appeared by the 18th day and glycosuria by the 22nd day. In those given cadmium stearate, both proteinuria and glycosuria started by the 30th day.

Leucine Aminopeptidase (LAP)

Cousins et al. (1973) have demonstrated that in pigs increased Cd dosing correlates with decreased LAP activity in the renal cortex. It has been suggested that a cause of Cd-induced renal dysfunction and associated low molecular weight proteinuria is the interference of cadmium with enzymes, such as LAP, which are necessary for the reabsorption and/or catabolism of proteins (Webb, 1979; Cousins et al., 1973; Vigliani, 1969). As noted previously, in the kidney the proximal convoluted tubule is the site of protein reabsorption and catabolism and also the primary site of Cd accumulation.

LAP is a proteolytic enzyme that catalyzes the hydrolysis of N-terminal residues from certain peptides and amides containing a free amino group. Enzyme activity is particularly favored when the N-terminal group is leucine. The catalyzed reaction is as follows:



$$R_2-\overset{H}{\overset{|}{N}}-\underset{\underset{R_1}{|}}{\overset{O}{\overset{\|}{C}}}-\overset{H}{\overset{|}{C}}-NH_2 + H-O-H \xrightarrow{LAP} R_2-\overset{H}{\overset{|}{N}}-H + H-O-\overset{O}{\overset{\|}{C}}-\underset{\underset{R_1}{|}}{\overset{H}{\overset{|}{C}}}-NH_2$$

(ammonia, amine, amino acid or peptide)

(amino acid, particularly L-leucine or L-alanine)

It is probable that LAP activity is associated with a group of closely related enzymes rather than with one single enzyme (Kachmar, 1970).

Early work by Himmelhoch (1969) showed LAP to be a zinc (Zn) metalloenzyme with a molecular weight of 300,000. He concluded that Zn was at or near the enzyme's active site based on the following experimental results: 1) dialysis of the enzyme to remove Zn or replace it with Cd resulted in loss of enzyme activity proportional to the amount of Zn removed; 2) replacement of Cd by Zn caused reactivation of the enzyme; and 3) agents that strongly complex Group IIb metals, such as Zn, caused inactivation of the native enzyme. Other in vitro studies have shown that Cd, Hg and Cu each inhibit the activity of LAP (Webb, 1979).

More recent work by Carpenter et al. (1973) showed LAP as having a subunit with a molecular weight of 54,000 and two separate metal binding sites. The "Zn specific site" binds tenaciously to Zn and is required for enzyme activity. The "activation site" binds Zn more weakly. Under laboratory conditions, Mn or Mg were made to replace Zn in the activation site to yield Zn-Mn and Zn-Mg enzymes having specific activities higher than that of the native enzyme. At physiological pH, Zn could not be displaced from the enzyme; therefore, the authors concluded that the native enzyme had the Zn-Zn form.

LAP has been demonstrated in bacteria, plants and animals and appears to be present intracellularly in many animal tissues. It is secreted by the mucosa of the small intestine and is active in protein digestion. Early researchers speculated that LAP could be hydrolytic, converting peptides and amides into amino acids for intracellular use; that it could be involved with formation of peptide bonds through the condensation of L-leucine with other amino acids; or that it could be involved in transpeptidation reactions (Goldbarg and Rutenberg, 1958).

Using histochemical techniques on fresh frozen tissue, Nachlas et al. (1960) demonstrated the localization of LAP in numerous organs and tissues from rats and humans. The LAP appeared to be contained within the cytoplasm, unassociated with recognizable intracellular organelles. In the kidney, there was intense LAP activity in the brush border of the proximal convoluted tubules. In contrast, the glomeruli and other nephron segments showed no activity. Enzyme activity was demonstrated in many other tissues, including the striated border of the columnar cells of the small intestine, liver parenchyma, bile canaliculi and ducts, pancreas (in man), bronchial epithelium, macrophages and polymorphonuclear leucocytes (in man).

LAP is found regularly in human urine and serum. The daily excretion of urinary LAP appears fairly constant for an individual, with men excreting approximately twice as much as women. Similarly, the serum enzyme levels for normal people are fairly stable, so that there is no correlation between age and enzyme activity (Goldbarg and Rutenberg, 1958).

Any condition, either intra- or extra-hepatic, that causes biliary obstruction would result in elevated serum LAP. Increased serum enzyme levels result from pregnancy and diseases such as carcinoma of the head of the pancreas, diffuse carcinoma, lymphoma and granuloma involving the liver, and acute pancreatitis causing obstruction of the common bile duct (Wolf and Williams, 1973). Early researchers (Goldbarg and Rutenberg, 1958) found that in humans, serum LAP could be normal, while urinary LAP was high. On the other hand, whenever serum levels were high, the urinary levels always were increased. It was suggested, therefore, that there was a tendency towards the maintenance of homeostasis of serum LAP and that the assay of enzyme activity in the urine reflected changes in tissue LAP with greater sensitivity than did the assay of enzyme activity in the serum. It was not known, however, whether the urinary LAP was derived from glomerular filtration or from tubular secretion.

More recently (Ono et al., 1968) it was found that contrary to previous theories, urinary LAP is qualitatively different from serum LAP. Further, increased urinary LAP is seen most often in people with renal disease or malignant tumors and is not necessarily accompanied by an increase in serum LAP. Levels of urinary LAP significantly higher than normal were found in people with nephritis, conditions involving renal tubular pathology, malignant tumors, acute inflammatory disease, liver disease, and diabetes.

Nephrosis, characterized by early proximal tubular damage, was induced in male Wistar rats by injection of $HgCl_2$. Acute tubular damage was accompanied by an immediate two- to 3.5-fold increase in urinary LAP above normal levels. Aminonucleoside nephrosis was induced in other male Wistar rats by injection of aminonucleoside. A significant increase in urinary LAP occurred during the second week after injection and corresponded to the delayed onset of the phase of the disease corresponding to renal tubular damage. In both cases, histochemical techniques showed LAP-positive material inside the dilated renal tubules and in the urine sediments (Ono et al., 1968).

Purified urinary, serum and renal cortical LAP were subjected to both cellulose acetate and starch zone electrophoresis. It was found that urinary and renal LAP migrated with identical mobility, but this differed from the mobility of the serum enzyme. Despite the small number of rats used (total of six), the authors concluded that urinary LAP was derived from renal LAP, and not directly from the serum and that increased urinary LAP was a sign of renal tubular damage (Ono et al., 1968).

Significant decreases in renal cortical LAP activity were seen in rats injected with $HgCl_2$ (Schulte-Wissermann, et al., 1977). This decrease was attributed to the destruction of proximal tubular epithelium and enzyme protein. Similarly, there is a significant depression of LAP activity in the renal cortex of rats and pigs that have been exposed to high doses of injected or ingested cadmium (Webb, 1979; Cousins et al., 1973). Vigliani (1969) also found a significant decrease in renal LAP activity in mice, following four daily endoperitoneal injections of 50 μg Cd^{+2}.

Cousins et al. (1973) found that although renal LAP activity in the pig was significantly reduced by Cd exposure, there was no affect on serum enzyme activity. They suggested, therefore, that Cd-bound proteins, such as metallothionein, found in the glomerular filtrate were reabsorbed in the proximal tubular cells and catabolized. The bound Cd was liberated in the tubular cells and free to exchange with the Zn of the tubular LAP and thus inactivate the enzyme and alter renal proteolytic activity.

These findings of Cousins et al. (1973) have been interpreted in another way. It has been suggested that diminished renal LAP activity is not the result of enzyme inactivation by Cd, but rather is due to actual loss of the enzyme through alterations in the membranes of the tubular brush border, similar to those described in Hg-intoxicated animals (Webb, 1979).

Formed Elements Exfoliated Into the Urine

Examination of the urine sediment provides useful information about the presence of renal disease, the nature of this disease, and prognosis during recovery.

In humans, the normal urine sediment may contain an occasional cast, a few renal tubular epithelial (RTE) cells, and epithelial cells from the bladder, ureter, urethra, and vagina. Mucous threads, spermatozoa, a rare erythrocyte, and up to four polymorphonuclear leukocytes may be seen per high power field (Kurtzman and Rogers, 1974; Lippman, 1957).

Cells in the urine can arise by desquamation of lining epithelium. Epithelial cells may originate from any site - from the proximal convoluted tubule to the urethra or vagina. Cells from any or all of these sites may be found in the urine as a result of normal attrition, with sloughing of old cells and growth of new ones. Any pathologic renal condition may result in increased sheeding of cells (Kurtzman and Rogers, 1974).

The appearance of increased numbers of RTE cells in the urine has great significance, since these cells degenerate and slough off in great profusion as a result of various renal diseases. This condition is common in acute tubular necrosis and may be accompanied by the appearance of RTE cell inclusion casts. Increased numbers of RTE cells also may be seen in malignant nephrosclerosis, acute glomerulonephritis, and pyelonephritis (i.e., infectious disease affecting the interstitial renal tissue and renal pelvis). Diseases characterized by heavy proteinuria also may yield clumps of RTE cells or RTE cellular casts (Kurtzman and Rogers, 1974; Koushanpour, 1976).

The presence of casts is of great diagnostic importance in the evaluation of urine sediments. Casts are pieces of gelled protein-like material that result from the agglutination of cells and the precipitation of protein present in the tubular lumen. Increased urine acidity and concentration, plus low urine flow in the distal nephron probably assist in cast formation. Casts are washed out of the tubule by flowing urine (Kurtzman and Rogers, 1974; Koushanpour, 1976).

There is a system of classification and nomenclature to identify the various types of casts based on their site of formation in the nephron, their consistency, and the type of cells (i.e., RBC, WBC, tubular cell, etc.) trapped in their matrix. As an example, inclusion casts are formed when protein in the tubular lumen gels and entraps any material, such as cells, present within the lumen. As the inclusion cast remains in the nephron, it undergoes a series of morphological changes due to the degeneration of the trapped cells. The cast goes through successive phases of "coarsely granular" to "finely granular" to "waxy." Each phase corresponds to further deterioration of the cellular material trapped within the matrix. A fresh cast contains intact cellular material that has not started to degenerate (Kurtzman and Rogers, 1974; Lippman, 1957).

The quantitative assessment of renal tubular cell excretion in the urine has been employed to detect tubular lesions induced experimentally by mercuric chloride ($HgCl_2$), uranyl nitrate, and other nephrotoxic agents (Diezi and Biollaz, 1979). Prescott and Ansari (1969) administered $HgCl_2$, a known proximal tubular nephrotoxin, to rats and studied renal tubular cell exfoliation in relation to histological evidence of tubular injury. Adult male Sprague-Dawley rats were given daily s.c. injections of 0.1, 2.0 or 4.0 mg $HgCl_2$/kg for up to 14 days. In all treated groups, except the 0.1 mg/kg group, there was a significant elevation in tubular cell counts that correlated with histological evidence of tubular necrosis. The cell counts rose rapidly to a peak and then fell at a slower rate despite continued exposure. The peak tubular cell counts coincided with the onset of massive tubular necrosis, and the subsequent drop in cell counts corresponded with diminishing tubular necrosis and increasing tubular regeneration, as the rats developed a tolerance to the $HgCl_2$.

Bonnell et al. (1969) injected male and female Wistar rats intraperitoneally (i.p.) with $CdCl_2$ at a dose of 1.0 mg Cd/kg, three times a week, for up to 45 weeks. Urine sediments from control and dosed animals were examined weekly and it was noted that excess red blood cells, and granular and cellular casts regularly were found in the urines of all dosed rats after one month of treatment.

Examining the urines from Cd-exposed workers, Schumann (1979) found a striking condition of "renal tubular injury." The findings included exfoliated renal tubular cells, pathological casts, increased crystaluria (i.e., crystals in the urine) and tubulo-interstitial inflammation presenting as white cell casts.

Specific Gravity and pH of the Urine

Specific gravity of the urine is the ratio of the weight of the urine to the weight of an equal volume of water under standard conditions. In man, the specific gravity of the glomerular filtrate is about 1.007. As this filtrate passes through the tubules and collecting ducts, the specific gravity is altered by tubular reabsorption or secretion of water and solutes (Becker et al., 1971). Because the specific gravity of the urine indicates the quantity of water relative to the solute that is reabsorbed from the glomerular filtrate as it flows through the tubules, it is used as a means of testing the concentrating and diluting abilities of the kidney (Koushanpour, 1976; Kurtzman and Rogers, 1974).

Specific gravity of normal human urine may range from 1.005 to 1.030 (Koushanpour, 1976), while that of normal rat urine may be 1.022 to 1.050 (Ringler and Lyubica, 1979). Proteinuria and glycosuria, each in concentrations exceeding 10 mg/ml, will increase the specific gravity of urine (Kurtzman and Rogers, 1974). In man, urine with a specific gravity greater than 1.010 indicates that concentration has occurred, whereas a specific gravity less than 1.010 indicates dilution (Becker et al., 1971).

In people on a normal diet, the inability to concentrate urine almost always indicates renal disease. The concentrating ability may be depressed as a result of many, varied problems including the action of certain drugs, nephrotoxic agents, or a variety of diseases, such as chronic renal infection and glomerulonephritis (Becker et al., 1971; Diezi and Biollaz, 1979; Koushanpour, 1976). Shiroishi et al. (1977) found that the average specific gravity of urine was lower in Itai-itai patients with chronic exposure to high dietary cadmium, as compared to normal controls and individuals with glomerulonephritis. There is also some evidence that in workers exposed chronically to high levels of Cd, the ability to concentrate urine is disturbed (Kazantzis, 1979b; Chang et al., 1981).

Several different mechanisms can be involved in the alteration of the kidney's urine concentrating ability. For example, the presence of a cortico-medullary osmotic gradient is a prerequisite for the production of hypertonic urine. Thus, renal changes which disturb this gradient also will impair the ability of the kidney to maximally concentrate the urine. Such changes include major depression of the glomerular filtration rate, disturbances of proximal tubule fluid reabsorption, and circulatory changes in the renal medulla. Any widespread functional kidney damage can result in a depressed urinary concentrating ability (Diezi and Biollaz, 1979).

The pH of the urine is a measure of the ability of the kidney to respond to disturbances in the acid-base balance of the body fluids. The elimination of hydrogen ions from the body, via the urine, is accomplished by their secretion into the proximal and distal tubules and collecting ducts. In the proximal tubules, protons are secreted in conjunction with the regeneration and reabsorption of bicarbonate ions. In the distal tubules and collecting ducts, additional protons are eliminated by combination with other buffers, primarily phosphates (Becker et al., 1971; Diezi and Biollas, 1979).

Usually, the urine is acidic because of the acidic metabolites produced during the normal breakdown of nutrients and body tissues. However, there is considerable variation in the pH of random urine specimens because of diurnal variations, diet and the presence of certain diseases. In man, the kidney can produce urine with a pH range of 4.5 to 8.0; however, the average normal value is 6.0 (Becker et al., 1971; Kurtzman and Rogers, 1974). Normal male Fischer 344 rats were found to have a urine pH range of 6.0 to 6.5 (Kluwe, 1981).

Persistently acidic or alkaline urine may result from certain systemic diseases and from diseases restricted to the kidneys and lower urinary tract. When the capacity of the tubules to secrete protons is depressed, the urine will be alkaline. The urinary pH cannot be reduced to the maximum extent when the net hydrogen secretion in the distal tubule is impaired (Diezi and Biollaz, 1979; Kurtzman and Rogers, 1974).

When proton secretion in the proximal tubules is reduced, bicarbonate reabsorption also will be reduced and the urine will be alkaline due to the abnormal excretion of bicarbonate. This condition is a prominent feature of the adult Fanconi syndrome (Kurtzman and Rogers, 1974). Although data concerning urine acidification in Cd-exposed humans and animals are generally lacking, Adams et al. (1969) concluded that such defects in acidification, as seen in the Fanconi syndrome, would be expected in at least some cases of tubular dysfunction resulting from occupational exposure to cadmium.

More recently, Kazantzis (1979b) described a worker who had been exposed for over 30 years to cadmium sulfide dust and for shorter periods to cadmium oxide fume and dust. The man exhibited many classical signs of Cd-induced nephropathy, including tubular proteinuria, glycosuria, aminoaciduria, and polyuria. Additionally, he had alkaline urine, which was confirmed by appropriate testing to result from an impaired tubular ability to acidify urine.

SECTION 3

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Two hundred, seventy-two male Wistar rats, aged 27 days at the start of the study, were obtained from Charles River Breeding Laboratories, Inc. The animals were divided into a Control and three experimental groups, with each group containing 68 rats. The groups were given cadmium chloride ($CdCl_2$) in the drinking water in concentrations of 0, 0.5, 5.0, and 50 mg per liter as Cd for up to 24 months. All animals were given water and commercial laboratory chow (Charles River - RMH 1000) ad lib.

Urinalysis was performed on one particular group of rats at 6 to 8 week intervals for the entire study. Every three months, eight rats from the Control and each of the dosage groups were sacrificed and various tissues, including the kidney and renal tubules, were analyzed for Cd, Zn and Cu. Urinalysis was performed on each rat the week prior to sacrifice.

The rats were housed two per cage in large plastic shoebox cages with a stainless steel wire grill covering the top of the cage. Each cage had a fibrous filter top to reduce transmission of airborne diseases. The cages were shelved on stainless steel racks, with a separate rack used for each dosage group.

The animal room was maintained at 78°F, 48% average relative humidity, and a 12-hour light/dark cycle.

Sick rats were separated from their box mates but were maintained on their regular Cd exposure schedule. With the exception of two rats, sick animals were not given medication, since drugs may have interfered with the study. Dead rats were autopsied and examined grossly for tumors. Moribund rats were humanely killed and autopsied. Tumors were preserved in 10% formalin and later examined microscopically.

DIVISION OF WORK INTO TWO PARALLEL STUDIES

Because of limited equipment availability and to make the project more manageable, all work was conducted as two parallel studies. To accomplish this, one half of all the animals (designated "early arriving" rats) were received in November and the other half (designated "late arriving" rats) were received exactly two weeks later, in December. Rats in both sets were the same age, i.e., 28 days old, at the time of their arrival.

In an attempt to randomize errors, such as inherent physiological differences between the two incoming groups of rats, the animals were segregated as follows: of the eight rats per dosage group that were assigned to each sacrifice date, four were taken from the early rats and four were taken from the late rats. All autopsies and urinalyses were staggered exactly two weeks apart to coincide with the difference in arrival times of the two groups.

PREPARATION OF DRINKING WATER CONTAINING CADMIUM

Three times a week, fresh water was prepared in separate 20 l polypropylene carboys and dispensed to the drinking bottles. Distilled water was used for the Control group and for preparing the water for each exposure group. Anhydrous $CdCl_2$ (Mallinckrodt Chemicals, Zn contamination 0.05%) was used to prepare the 50 mg/liter Cd carboy and the other test groups were prepared as dilutions from this. The $CdCl_2$ was dried overnight at 110°C. To ensure that each experimental group was given the proper water, each group was housed in a separate rack and the appropriate carboy was set on top of the rack, and water was dispensed from it. Each cage had a 500 ml polyethylene drinking bottle outfitted with a rubber stopper and a stainless steel "sippin" tube designed to reduce significantly the amount of water lost through leakage.

The concentrations of Cd, Zn and Cu in the freshly prepared carboys were checked immediately by flame atomic absorption spectrophotometry (AAS). Any batch of water whose Cd concentration was not close to the desired value was discarded and another batch was made. The pH also was monitored. Additionally, the Cd levels of randomly selected drinking bottles were checked, roughly once a week. Table 3-1 gives the range of values obtained for the Cd concentrations and pH of all the batches of prepared water.

Table 3-1. Concentrations of Cd and pH of the Prepared Water

Dosage Group	Actual Cd Concentration Mean (mg/l) ± S.D.	pH
Control (0.0 mg/l Cd)	0.00 ± 0.00	5.05 - 5.85
Group II (0.5 mg/l Cd)	0.514 ± 0.06	4.70 - 5.40
Group III (5.0 mg/l Cd)	5.09 ± 0.49	4.40 - 5.20
Group IV (50.0 mg/l Cd)	50.29 ± 3.67	4.30 - 5.00

Zinc and copper were never detectable.

Since Cd is absorbed out of solution by plastics and glass, it was important to acid-wash the containers and frequently change the dosed water (U.S. Dept. Health, Education, Welfare, 1976). Through preliminary work, it was determined that acid-washing the carboys and drinking bottles approximately twice a month and replacing the contents of the drinking bottles with freshly prepared water three times a week (i.e., Monday, Wednesday, Friday) was adequate to maintain the desired levels of Cd in the drinking bottles. Acid-washing was accomplished by soaking the containers overnight in 15% HNO_3 and then thoroughly rinsing them in distilled water.

CONCENTRATION OF CADMIUM, ZINC AND COPPER IN THE RAT FOOD

Samples from new bags of rat chow routinely were tested for Cd, Zn and Cu. Duplicate 1 gram samples of food from each bag were digested in concentrated nitric acid and H_2O_2 and assayed in a manner similar to that used for assaying tissue metals (see subsection on Methods for Assaying Tissues for Cd, Zn and Cu). For the whole study, the concentrations of metals in the food were as follows:

	Mean ± S.D. (μg/g wet weight)
Cd	0.272 ± 0.263
Zn	101.910 ± 22.85
Cu	8.970 ± 2.17

DATA COLLECTION

To keep track of the large volume of data collected for each rat, each animal was assigned a specific number. To reduce experimental bias, animals were randomly drawn from the shipping crates and immediately assigned a number and a sacrifice date. Each rat number had two parts. The first part was the experimental group designation (i.e., Control, Group 2 = 0.5 mg/l; Group 3 = 5.0 mg/l; Group 4 = 50 mg/l Cd) and the second part was the individual number designation within the group (i.e., 1-68). Each rat's toes were clipped according to the code shown in Figure 3-1, so that each animal was permanently numbered (Kumar, 1979). There were no problems with toes regrowing and obliterating the numbers. Additionally, each rat was assigned to a numbered box and a similarly numbered water bottle.

Each rat was weighed once a week in a top loading balance. Food and water consumption were measured for each rat box (i.e., containing two rats). Food, in the form of large, solid pellets stored in the wire grill on top of the box, was easily poured out and weighed. Any food falling into the box also was weighed so that inaccuracies in weights due to food loss were negligible. Food consumption was measured once a week.

Water consumption was measured three times a week when fresh water was prepared. Each water bottle was weighed, emptied, refilled and reweighed. This method resulted in an insignificant amount of water lost by spilling

Autopsy Data

Every three months, eight rats from each group were sacrificed, various organs removed, and tests performed as indicated in Table 3-2. Each rat was weighed and injected with pentabarbital (65 mg/ml) at a dose of 0.1 cc/100 grams body weight. While the rat slept, the abdomen was opened and heparin was injected into the dorsal aorta at a dose of 0.1 cc/100 grams body weight. Shortly thereafter, a polyethylene cannula was inserted into the aorta and blood was drawn off into a clean, 15 ml graduated, conical centrifuge tube. The blood was centrifuged at 1,900 rpm for 25 minutes. The volumes of whole blood and packed red cells were recorded so that the hematocrit could be determined, as the percent of packed red cells.

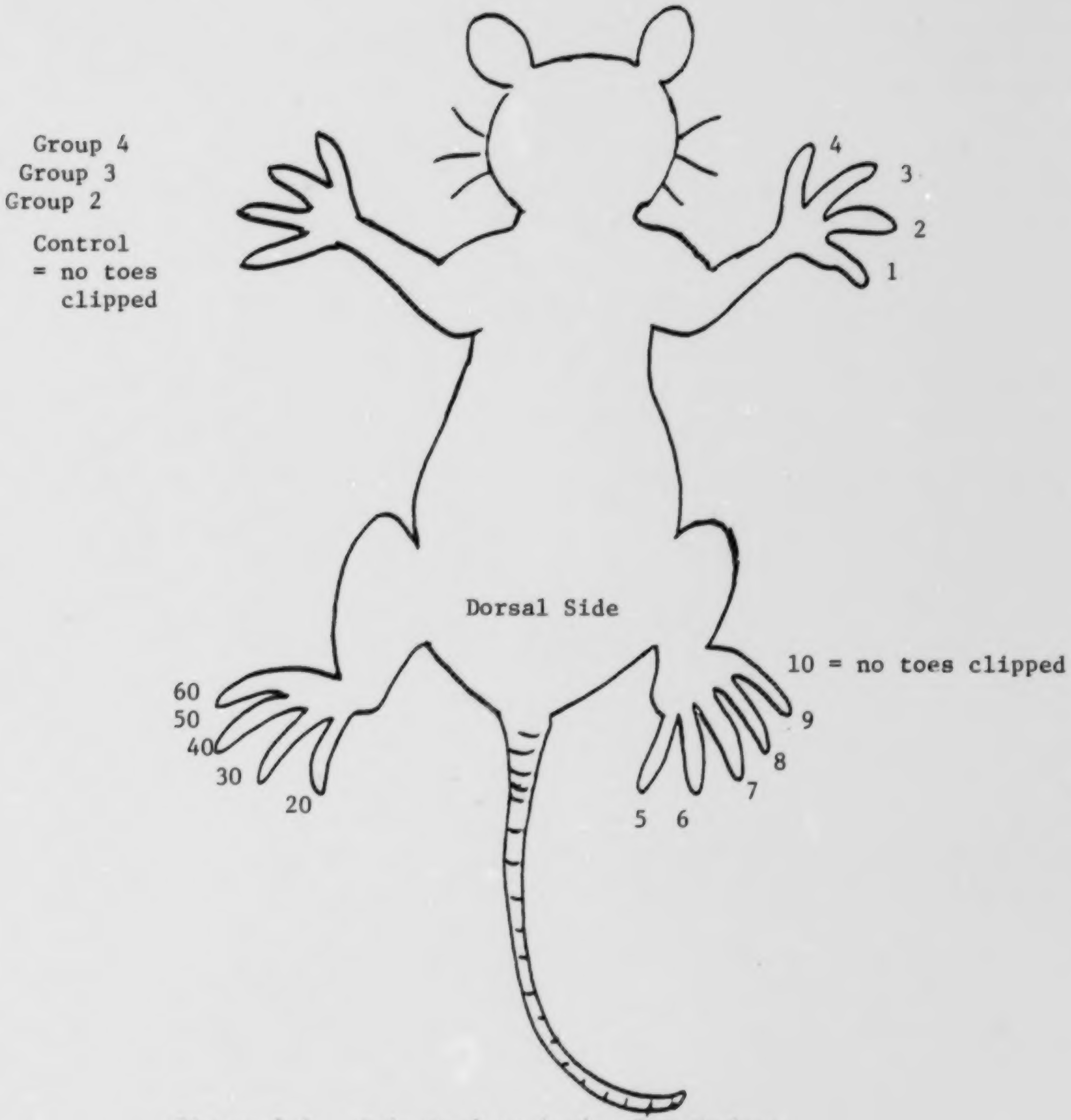


Figure 3-1. Code Used to Assign Rat Numbers

Table 3-2. Data Collected at Autopsy

Organ or Tissue	Organ weight		Cd, Zn Cu	Histology	Other tests
	Wet	Dry			
Whole kidney	X			X	LAP*
- outer cortex	X	X	X		
- cortical tubules	X	X	X		
Liver	X	X	X		
Muscle	X	X	X		
Small intestine	X	X	X		
Spleen	X	X	X		
Heart	X	X	X		
Lung	X	X	X		
Blood					Hematocrit
Tumors				X	

*LAP is the enzyme leucine aminopeptidase.

Tumors found on gross examination were excised, and their sizes, locations and appearances were recorded. Tissues were preserved in 10% formalin and sent to the Department of Comparative Medicine for examination.

Preparation of Tissues for Metals Assay

To prevent contamination of tissues with extraneous heavy metals, all plastic and glassware used during the sacrifice were acid washed, dried, and kept in covered, aluminum foil-lined baskets. All acid washing was accomplished by soaking the article overnight in 15% HNO_3, removing and thoroughly rinsing it in deionized, distilled water, and drying it in an oven. Additionally, only deionized, distilled water was used during the sacrifice to rinse organs, glassware, and surgical instruments.

The acid bath solution, contained in a large, covered polypropylene box, was checked weekly by flame AAS for levels of Cd, Zn and Cu contamination. A fresh solution was prepared whenever any metal reached 0.05 ppm concentration.

During the sacrifice, all organs (see Table 3-2) except the kidney, were removed, rinsed in a stream of deionized, distilled water and placed into preweighed, acid-washed, polystyrene snap-cap vials. Since the fecal material contained significant concentrations of heavy metals, the small intestine was removed, slit open along its entire length and carefully scraped to remove all feces. The tissue was rinsed thoroughly in water, patted dry on paper towels and placed into a weighing vial. Each filled vial was weighed on a Mettler balance to obtain the total wet weight of each organ.

To prepare each organ for metals analysis, roughly 1 to 2 grams of fresh tissue were removed, weighed, placed in a clean, preweighed aluminum weighing pan and dried for 24 hours in a 110°C oven. After drying, the pan and tissue were cooled in a dessicator and reweighed to obtain the dry weight. This dried tissue was used for determining the concentration of heavy metals, as will be described later.

Unlike the other organs, four different procedures were performed on the kidneys. The right kidney was sliced in half longitudinally and one-half was used for histology, while the other half was used for the assay of the enzyme leucine aminopeptidase (LAP). The left kidney was used for the metals assay. In order to compare the metals concentrations in the cortical tubules accurately with that of the intact cortex, only one kidney, the left one, was used to obtain both tissues. This kidney was sliced in half longitudinally, and one-half was used to extract the tubules, while the other half was used to obtain the intact cortext. Table 3-3 outlines the procedures used to obtain both the intact cortex and the tubule preparations.

Preparation of the cortical tubules was adapted from the methods of Dawson (1972). After the kidney was sliced in half, the piece used for tubule extraction was placed immediately in ice cold buffered saline. The buffered saline was prepared according to Dawson's methods with the exception that the normal saline (0.9% NaCl), which comprised 93.5% of the final volume of the buffered solution, was first run through a Chelex column to remove heavy metal

Table 3-3. Procedures for Preparing the Intact Renal Cortex and Cortical Tubules Used for the Assay of Cd, Zn, and Cu

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Left Kidney
Slice in Half

Whole Cortex Preparation	Tubule Preparation
1. Remove outer cortex	1. Remove outer cortex
2. Weigh	2. Chop cortex
3. Dry overnight - 110°C	3. Digest at 37°C in 0.05% collagenase + 0.1% hyaluronidase in buffered saline*
4. Weigh	4. Filter through white nylon stocking and gauze
5. Digest - nitric acid; H_2O_2	5. Centrifuge filtrate - 700 rpm in 30 ml, acid-washed polyethylene centrifuge tube
6. Analyze - Cd, Zn, Cu	6. Wash pellet in buffered saline several times to remove enzymes
	7. Dry overnight - 110°C
	8. Weigh pellet
	9. Digest - nitric acid
	10. Analyze - Cd, Zn, Cu

*Collagenase Type I and Hyaluronidase Type I-S supplied by Sigma Chemical Company.

contaminants. The metal-free saline was then mixed with the remaining salts, the pH was adjusted to 7.4, and the final solution was checked for Cd, Zn and Cu contamination using flame AAS. These metals were never found in detectable quantities. Preliminary work showed that running the finished buffer through the Chelex column resulted in altering the buffer, such that the tubule preparations were ruined and no tubule segments could be recovered. On the other hand, when only the NaCl solution was run through the Chelex column, the buffer could be effectively decontaminated of Cd, Zn and Cu, and its chemical composition was preserved.

With this method each half kidney usually yielded 2-10 mg of dried tubules. Figure 3-2 shows the microscopic view of a typical unstained tubule preparation at low power. Each pellet was examined microscopically prior to being dried. A good pellet consisted of short tubule segments with few glomeruli and little cell debris. Depending on the quality of the preparation, upwards of 90% of the material examined microscopically was of a size consistent with proximal tubules.

Methods for Assaying Tissues for Cd, Zn and Cu

Only dried tissues were used for the metals assay and all were handled in a similar manner. Briefly, the tissue was placed in a 100 ml, acid-washed Kjeldahl flask to which 20 ml of concentrated HNO_3 were added. The mixture was gently boiled for three to four hours until all the tissue was digested and the liquid was a clear yellow. The flask was cooled and 4 ml of 30% H_2O_2 were added. The mixture was reheated at a gentle boil for one hour and then cooled. The contents of the flask were transferred to an acid-washed 25 ml volumetric flask. The Kjeldahl flask was rinsed several times with deionized, distilled water and the washings were used to fill the volumetric flask to the mark.

This basic procedure was modified to prepare the renal tubule and fecal samples. Due to the small sample size of the tubules (i.e., 2 to 10 mg dried tissue), the digestion process was conducted in the polyethylene centrifuge tube used to prepare the tubule pellet (see Table 3-3). Only 5 ml of concentrated HNO_3 were used and the H_2O_2 was omitted. The final volume of the digested tubules was 10 ml instead of 25 ml. Because of the harsh digestion process, each polyethylene centrifuge tube could be used only one time.

In preparing the fecal samples, approximately 0.5 to 0.8 gram of dried feces was placed in a 100 ml acid-washed Kjeldahl flask and digested with HNO_3 and H_2O_2 in the same manner as outlined for the nontubular tissues. At the end of the digestion process, a significant amount of nondigestable, inorganic matter remained. These particles had to be removed or they would have clogged the atomic absorption spectrophotometer. To accomplish this, the cooled contents of the Kjeldahl flask were poured through a glass fiber filter (Whatman GF filter) and then into the 25 ml volumetric flask. Further, because of the high concentrations of fecal Cd and Zn, the contents of the volumetric flasks were diluted 1:10 before running them on the AAS.

With every set of tissues digested, a blank and a National Bureau of Standards (NBS) standard were run. The blank consisted of 20 ml concentrated HNO_3 plus 4 ml of 30% H_2O_2. Additionally, the blank for the fecal samples was filtered through a Whatman GF filter and then poured into the volumetric flask.

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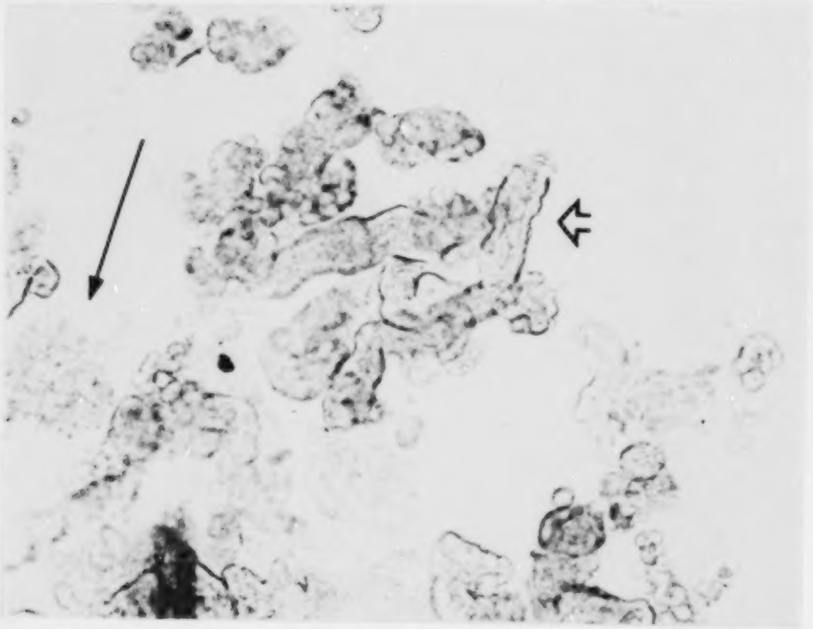


⇧ indicates cortical tubules segments

⬆ indicates glomerulus

Figure 3-2. Low power, light microscopic view of unstained preparation of isolated cortical tubules taken from a one year old rat. (110 magnification).

The NBS standard, made from bovine liver, was lyophilized overnight and 300 to 500 mg of weighed material were digested. Table 3-4 gives certified values of each metal and the range of values that were obtained during the study.

Table 3-4. Range of Metals Values for the Certified NBS Standards for Bovine Liver Cd, Zn, Cu

	Certified Value (μg/g dry weight)*	Values for the Study (μg/g dry weight)
Cd	0.27 ± 0.04	0.28 ± 0.06
Zn	130 ± 13	123.7 ± 4.61
Cu	193 ± 10	188.3 ± 6.34

(*Values obtained from the National Bureau of Standards, U.S. Department of Commerce, Certificate of Analysis for Standard Reference Material 1577, bovine liver.)

The concentrations of metals in all the digested tissue samples and water samples were determined by using a Varian Techtron (Model AA-5) flame atomic absorption spectrophotometer equipped with a strip chart recorder. The concentrations were calculated from the peak heights. The sensitivities of the instrument for the three metals were: Cd, 0.011 μg/ml; Zn, 0.009 μg/ml; and Cu, 0.04 μg/ml.

Procedures for Assaying Renal Leucine Aminopeptidase (LAP)

Half the right kidney from each rat was used for determining the levels of the enzyme LAP. This kidney was removed, sliced in half longitudinally, and one half immediately was put in ice cold 0.001 M Tris-HCl buffer, pH 7.4. The outer cortex was removed, blotted dry, weighed and then the methods of Cousins *et al*. (1973) were used to extract the enzyme. First, a 5% homogenate of the cortex in Tris buffer was made by grinding the tissue in a glass homogenizer with a motor driven Teflon rotor. The homogenate was centrifuged in a Sorvall ultracentrifuge at 42,000 g for 2 hours at 4°C.

LAP activity was determined on an aliquot of the supernatant by measuring the liberation of β-naphthylamine from the substrate, L-leucyl-β-naphthylamide. This colorimetric assay is a modification of the original Goldbarg and Rutenberg (1958) procedure and was carried out with a test kit supplied by Sigma Chemical Company. The reaction system is as follows:

1. L-leucyl-β-naphthylamide + $H_2O \xrightarrow{H+}$ L-leucine + β-naphthylamine
2. β-naphthylamine + $NaNO_2 \xrightarrow{H+}$ Diazo Reagent
3. Diazo Reagent + N-(1-naphthyl)-ethylenediamine $\rightarrow$ Blue Azo Dye

The absorbance of the Azo dye follows Beer's law and is proportional to the β-naphthylamine concentration, which in turn is proportional to the original LAP activity.

The Sigma (1977) procedure for determining LAP activity in serum was used to measure renal LAP. An aliquot of the cortex supernate was diluted 75-fold with distilled water and assayed. For each kidney, three reaction tubes were set up as follows: 1) reagent blank = 0.5 ml water + 0.5 ml substrate, 2) kidney blank = 0.5 ml water + 0.5 ml diluted cortex supernate, 3) test mixture = 0.5 ml substrate + 0.5 ml diluted cortex supernate. The tubes were incubated for one hour at 37°C. The reaction was stopped by the addition of HCl. Successive addition of sodium nitrite, ammonium sulfamate, and an alcoholic dye base completed the reaction. After 45 ± 10 minutes (at room temperature), the absorbance was read at 580 nm using a Spectronic 20 spectrophotometer. A standard curve was generated using the Sigma LAP calibration solution, consisting of 1.8 mg β-naphthylamine/100 ml in acid solution.

To monitor the assay procedure, a Sigma enzyme control was run with each set of unknowns. The acceptable range of values for the controls and the range of values produced in the laboratory were as follows:

	LAP Activity - Sigma units/ml*	
	Sigma Values	Study Values
Sigma Control 2-E (elevated range)	3.7 ± 0.37	3.75 ± 0.23
Sigma Control 2-N (normal range)	1.2 ± 0.12	1.33 ± 0.14

*A Sigma unit will release 1 mole (143 μgm) of β-naphthylamine from the substrate per hour at 37°C at pH 7.1.

It was found that this enzyme assay had to be performed on fresh kidney tissue, preferably as soon as possible after centrifugation of the homogenate. Overnight storage of the cortical supernatant at -20°C resulted in significant loss of LAP activity. Presumably, other renal enzymes in the supernatant degraded the LAP.

Histochemistry

At autopsy, half the right kidney was reserved for histology. The half kidney was removed and immediately placed into a buffered formalin solution (4% paraformaldehyde, 2% calcium acetate, pH 7.1) to fix for 24 hours. The tissues were rinsed briefly in cold tap water and then run through the following solutions: 70% ETOH (overnight), 90% ETOH (2 x 1 hr), 100% ETOH (2 x 2 hrs), xylene (3 x 1 hr), cedar wood oil (until paraffin embedding). The tissues were soaked in toluene for one hour and then infiltrated with liquid paraffin. (Tissue Prep, melting point 56.5 ± 0.5°C, Fisher Scientific Co.). The infiltration process consisted of 3 x 1 hr changes of liquid paraffin and was conducted in a vacuum oven (60°C, ⁻20 psi). The infiltrated tissues were embedded in individual blocks of paraffin.

All tissues were sectioned at 4 μm and the prepared slides were labelled with individual rat numbers. To avoid observer bias, the number on each slide was covered with paper, the slides were mixed up and then drawn at random for examination.

In order to assess sequential morphological changes, sections from the kidney of each rat were treated with three separate stains. First, routine hematoxylin and eosin (H & E) was used for the identification of cell types and structures and the diagnosis of certain pathological changes. Periodic Acid Schiff (PAS) was used to identify proximal convoluted tubules by their bright pink staining brush borders. PAS also was used to visualize glomerular basement membranes. The Masson Tri-chrome stain was used to identify the interstitial connective tissue, which stains a bright blue (Luna, 1968).

In order to establish a pattern of normal renal aging, and renal aging altered by Cd dosing, various structures in the kidneys of the control rats and each of the three Cd-dosed groups were evaluated morphometrically. Well established morphometric techniques were used in all evaluations (Visscher and Stifano, 1981; Weibel, 1969; Bolender, 1978). Such techniques routinely are used for the analysis of biological tissue samples and for the interpretation/quantitation of structural changes, and the assessment of structure versus function correlations.

For this work, an eyepiece grid (100 mm^2 divided into blocks of one mm^2) was used in the microscope. The grid had a total of 121 test points, and a test line of 2.75 mm (at 40X magnification). For each kidney, measurements were repeated at three separate fields. The fields, located in the outer cortex, were taken at either kidney pole and midway between the two poles. The areas to be examined were first marked with a felt-tipped pen so that roughly the same area would be examined for each slide. The outer cortex alone was used for the morphometric analysis; however, the entire kidney section was examined for pathological changes.

Although it was not possible to estimate the total number of glomeruli per kidney, three other measures (Weibel, 1969) were used as relative indicators of age- and Cd-related changes in the population of glomeruli. The measures are as follows:

1) Volume density of glomeruli (Vv). This parameter, calculated by point counting of the glomeruli at 10X, represents the total volume of glomeruli per unit volume of test grid.

$$Vv = \frac{\text{number of test points lying within the glomeruli}}{\text{total number of test points in the grid}}$$

2) Numerical profile density on area of the glomeruli (Na). This parameter represents the total number of glomerular profiles per unit area of a random tissue section. It can be expressed as the number of glomeruli/mm^2 of outer cortex. Na was determined using the scan objective and counting the total number of glomeruli per test grid.

3) Numerical density of the glomeruli (Nv). This parameter estimates the number of glomeruli per unit volume of cortex. Unlike Na, Nv takes into account the size of the glomeruli, which is important since they grow larger as a function of age. A rough estimate of Nv was made using the formula of Weibel (1969) as follows:

$$Nv = \left(\frac{K}{B}\right) \bullet \frac{Na^{2/3}}{Vv^{1/2}},$$

where K = 1, because the glomeruli in any one kidney are assumed to be of roughly equal size, and B = 1.38, because the glomeruli are assumed to be roughly spherical in shape.

Also, the sizesof the glomeruli were determined. Using a 40X objective, 5 glomeruli per field, or 15 glomeruli per kidney section, were measured. The glomeruli were randomly selected and the long and short diameters were measured using the eyepiece grid. For each dosage group at each sacrifice, the long, short, and average diameters (i.e., long+short diameters/2) were calculated for the group pooled data.

To provide a relative measure of the change in the amount of healthy proximal tubular tissue, the Surface Density (Sv) was calculated. Using a 40X objective, Sv was calculated by counting the number of times a proximal tubule wall crossed any of the horizontal lines of the test grid. Then:

$$Sv = \frac{\text{number of tubule crossings per test line}}{\text{total length of test line}}$$

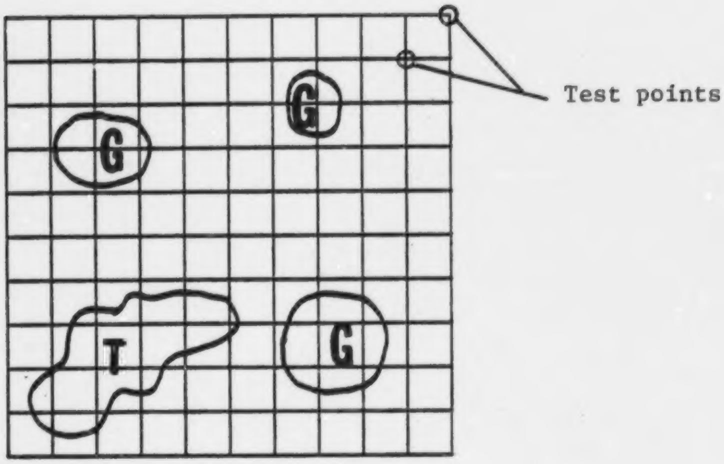
To illustrate the manner in which the morphometric analysis was conducted, a test grid containing three glomerular and one tubular profile has been provided in Figure 3-3, along with sample calculations of Vv, Na and Sv.

Finally, to assess the pathological changes in the kidney, tissue characteristics were examined as follows: inflammation, interstitial fibrosis, hyperplasia, tubular dilation/cast formation, and thickening of the glomerular and proximal tubular basement membrane. Each slide was scanned at low power to get an overall view of the extent of tissue involvement, then high power (40X) was used to focus on appropriate details. Tissue damage was ranked as 0 = none, 1-2 = mild, 2.5-3.5 = moderate, and 4-5 = severe.

Urinalysis

Urinalysis was performed on each rat the week prior to its sacrifice. Additionally, for the duration of the study, urinalysis was performed every six to eight weeks on the eight rats from each group that were scheduled for the last sacrifice. The test rats were housed individually in stainless steel metabolic cages. While in these cages, all rats were maintained on their regular Cd dosing regime.

Each cage (Fig. 3-4) had a 100 ml, graduated glass drinking bottle which was acid-washed prior to each use. The bottles had stainless steel "sippin" tubes to reduce the volume of water lost through leakage. As shown in Figure 3-4, the food cup was suspended from an enclosed passageway located outside of the cage in order to minimize the amount of food spilled and dropped into the urine collection flask. The food was powdered laboratory rat chow. The regular pelleted chow used throughout the experiment was ground in a Waring blender to produce this powder.



Test grid has a total of 121 test points and an area of 100 mm^2

G = glomerulus

T = tubule

Length of test line = total length of all horizontal lines = 2.75 mm (at 40X). Volume density of glomeruli (Vv) =

$$\frac{\text{number of test points within all glomeruli}}{\text{total number of test points}}$$

$$= \frac{7}{121}$$

Numerical profile density of glomeruli (Na) = number of glomeruli/grid

$$= 3/100^2 \text{ mm}^2$$

Surface density of tubule (Sv) = (number of times tubule wall crosses horizontal test line) ÷ (total length of test line)

$$= 6/2.75 \text{ mm}$$

Figure 3-3. Test Grid and Sample Calculations Used in Morphometric Analysis of Renal Cortical Tissue

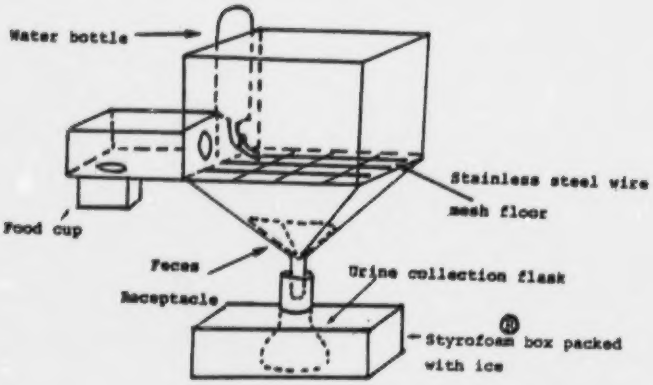


Figure 3-4. Metabolic Cages Used During Urinalysis

Feces were collected in a stainless steel cup located under the cage (Fig. 3-4). The cups were soaked overnight in EDTA and then rinsed thoroughly in deionized water in order to remove contaminant heavy metals.

Urine was collected in clean, 50 ml Erlenmeyer flasks. The flasks were set in leak tight, covered styrofoam boxes which were packed with plastic bags filled with crushed ice. In addition, each box contained a temperature holdover device to reduce melting of the ice. The device, known as "Blue Ice," and available commercially in department stores, was frozen overnight at -20°C prior to use. The boxes were designed to enclose the Erlenmeyer flask about 1/2" below the mouth and to keep it well packed with ice. In this manner, urine could be kept ice-cold for at least 16 hours.

Rats were weighed immediately before and after their stay in the metabolic cages. The environment of these cages was significantly different from that of the plastic shoebox cages in which they were normally housed. For this reason, all rats were acclimated in the metabolic cages for 48 hours during which time no samples or data were collected. After acclimation, food and water consumption were measured and urine and feces were collected over three consecutive 24-hour periods. It was important to collect samples over a 24-hour period as the amount of urine and feces produced varied throughout the day and night.

During each of the three sample collection days, the volume, pH and specific gravity of the urine were measured. The urine parameters assayed were: 1) day one - total protein, 2) day two - enzyme leucine aminopeptidase, 3) day three - glucose. All tests were performed individually for each rat. Pooled urine samples were never used.

The amount of feces excreted by each rat was measured separately for each of the three sample collection days. A composite sample prepared for each rat during the three days was used later for determination of the levels of Cd, Zn, Cu. To accomplish this, the total daily fecal output from each rat was placed in a preweighed, acid-washed, polystyrene snap-cap vial. The filled vial was weighed on a Mettler balance. To prepare the composite sample, each rat was assigned a clean aluminum weighing pan. For each of the three days, approximately 0.4 gram of fresh feces from each rat was weighed and placed in the appropriate pan. During the three days, these samples were kept covered in the refrigerator. At the end of the three days, all the samples were dried for 24 hours at 110°C, then cooled in a dessicator and weighed on a Mettler balance. The dried samples were used for the determination of metals as previously described.

METHODS FOR ASSAYING URINE PARAMETERS

pH, Specific Gravity, Refractive Index

The pH was measured by placing a drop of fresh urine on pH paper. The specific gravity was measured by using a hand held refractometer (model 100/C, National Instrument Co., Inc., Baltimore, Md.). A single drop of fresh urine was placed in the device, and the specific gravity was read directly from the appropriate scales. The scale for specific gravity was 1.000-1.045 (with 0.005 divisions) and that for refractive index was 1.3330-1.3600 (with 0.0001 divisions).

Total Protein

The methods of Piscator (1962) and Piscator and Pettersson (1977) were used to determine the total protein (TP) content of the 24-hour urine samples. The method is based on the biuret reaction after precipitation of protein with phosphotungstic acid in a hyrochloric acid-alcohol solution. The principle of the biuret reaction is that substances, such as proteins, containing two or more peptide bonds will form a purple complex with copper salts in an alkaline solution (Layne, 1957).

The reagents used were as follows:

1) Tsuchiya's reagent: 15 g phosphotungstic acid, 50.6 ml concentrated HCl, 770 ml 95% ETOH, 60 ml distilled water

2) 3% NaOH

3) Benedicts' reagent: 173 g sodium citrate and 100 g sodium carbonate were dissolved in 500-600 ml distilled water, and then filtered; 17.3 g copper sulfate ($Cu_2SO_4 \cdot 5\ H_2O$) were dissolved in 100 ml distilled water. The two resulting solutions were mixed and diluted with distilled water to make a total volume of 1 liter.

The original procedure was modified during the study in that centrifuged urine was used instead of filtered urine. During preliminary work, it was found that the rat urine contained substances, such as food particles, that quickly clogged the filter. As an alternative, several milliliters of urine were centrifuged at 1,900 RPM (CRU-5000 centrifuge, IEC) and the supernatant was used for the TP assay. To check this method, aliquots of urine from several rats were either filtered or centrifuged and then assayed for TP. No significant difference in protein concentrations were found between the two methods.

For the assay, 1 ml of diluted, centrifuged urine was mixed with 1 ml of Tsuchiya's reagent, allowed to stand at room temperature for 20 minutes, then centrifuged at 4,000 RPM for 20 minutes (Sorvall centrifuge, RC2-B). The pellet was washed once with 95% ETOH and recentrifuged. The pellet was then dissolved in 4 ml of 3% NaOH. Benedict's reagent (0.2 ml) was added and the mixture was allowed to stand at room temperature for 20 minutes. The absorbance was read at 330 nm using a Guilford spectrophotometer (model 240, Oberlin, Ohio) with a digital readout.

Each sample was run in duplicate. The blank consisted of 4 ml of NaOH + 0.2 ml Benedict's reagent. Crystallized human albumin (Miles Laboratories, Elkhart, Ind.), reconstituted in distilled water, was used to generate the standard curve. The assay was monitored by using diluted urine samples spiked with aliquots of albumin standard. In every case, there was excellent agreement between the calculated value and the actual test value.

Although there are several methods available for determining total protein, this procedure was selected for three main reasons. First, it is a sensitive assay that has been used successfully in several studies dealing with Cd-induced proteinuria (Axelsson and Piscator, 1966; Piscator, 1962; Piscator and Pettersson, 1977; Kojima et al., 1977; Nomiyama et al., 1980; Shiroishi et al., 1977). Second, it appeared to be the method of choice for the quantitative determination of urine total protein (Nomiyama, 1981; U.S. Dept. of Health, Education, and Welfare, 1976). Third, in contrast to other assays, the Tsuchiya-biuret assay precipitates all proteins in the urine (Piscator and Pettersson, 1977).

Glucose

Total urinary glucose was determined by using the test kit "Trucose Glucose" (American Monitor Corp., Indianapolis, Ind.) which is designed for quantitative determination of glucose in urine, serum and plasma. The reaction involves the condensation of an aromatic amine (O-toluidine) and the terminal aldehyde group of the glucose molecule to produce a blue-green product. The reaction is specific for aldohexoses and the absorbance at 630 nm of the final product is directly proportional to the glucose concentration of the sample. The sensitivity of this procedure is approximately 0.7 mg/dl measured by an absorbance change of 0.001.

Since bacteria rapidly degrade glucose, a preservative, 1% sodium azide (0.1 ml/10 ml urine) was put into each urine collection flask at the start of the collection period. Bacterial activity was sufficiently retarded through the combined use of the sodium azide and maintenance of the collecting urine at ice cold temperatures.

An aliquot of the urine was centrifuged at 1,900 RPM for 15 minutes to remove debris, such as cells and food particles. Since protein does not interfere with the assay, there was no need to precipitate out the protein. For the assay, 50 μl of centrifuged urine and 6.0 ml of "Trucose" reagent were mixed and placed in a boiling water bath for 10 minutes. After cooling the reaction mixture, the absorbance was read at 630 nm in a spectrophotometer (Spectronic 20, Bausch & Lomb).

A standard curve was prepared using anhyrous glucose in distilled water in a range of 1.00-4.00 mg/ml. As a quality control, aliquots of a 50% glucose standard (Combined Urea, Nitrogen and Glucose Standards, Fisher Scientific Co.) were run with each set of urines. The standard contained 0.50 mg/ml. The mean and standard deviation of values derived in this study were 0.49 ± 0.045 mg/ml.

Leucine Aminopeptidase (LAP)

The assay for LAP in urine was the same as that previously described for assaying LAP in the renal cortex, with the following exceptions:

1. An aliquot of the 24-hour urine specimen was dialyzed (dialysis sack #250-7U, Sigma Chemical Co.) against cold running tap water overnight. This removed chromogens which interfere with the assay.

2. The dialyzed urine was diluted 1:15 with distilled water prior to the assay.

Statistical Analysis

All data on animal weights, urine and fecal parameters, tissue metals, autopsy findings, etc., were quantified individually for each rat and converted into mean ± S.E. values for each group.

Since the primary objective of this study was to identify age-related versus Cd-related alterations in the kidney, analysis of variance (ANOVA) was performed on each parameter. The Scheffe procedure, which is a multiple range test, was used in conjunction with the ANOVA as a test for significance. With this method of analysis, it was possible to compare physiological and structural characteristics among the Control and three Cd exposure groups to determine whether there were significant differences and whether those differences were related to age, to Cd exposure, or to a combination of age and Cd exposure.

Additionally, Bartholomew's test for ordered means (Shirley, 1979) was used to examine the data pertaining to urine total protein, glucose, and volume and the data resulting from the histochemical examination of the fixed renal tissue. This is a powerful test that can be used for the statistical analysis of toxicological data which demonstrate a dose-response relationship.

For some of the data, particularly that dealing with metal kinetics, it was necessary to compare rates of change between the groups. For example, the rates of cortical versus cortical tubular Cd accumulation were compared to determine if there were significant differences between these two tissue types. For this kind of analysis, the slopes of appropriate graphs were computed using linear regression. Significant differences between the slopes were assessed by using the Student's t-test.

All calculations of mean values, standard error, linear regressions, and slopes were performed with a Texas Instruments desk calculator. All tests of significance involving ANOVA and t-tests were performed using an SPSS packaged computer program.

SECTION 4

RESULTS

As indicated in the Materials and Methods section, the entire study population was divided in half, with the first group of animals arriving two weeks before the second group. At each sacrifice point, 8 rats from each dosage group (i.e., Control, Group 2, Group 3, Group 4) were examined. In each group of 8 rats, 4 were early arrivals and 4 were late arrivals.

At the time of arrival, the early and late rats were of identical age. The animals were weighed and immediately assigned a sacrifice date. There were no statistically significant differences in initial body weight between the early and late rats within any of the dosage groups as a whole or within any of the dosage groups at a particular sacrifice point.

Since these were outbred rats received from the same breeding colony, it was expected that there would be random variations in the urine and tissue parameters, but that these parameters would be fairly similar between the early and late arriving rats. Thus, it was assumed that the early and late arriving rats could be treated as a single study population. To validate this assumption, a 2-way analysis of variance (ANOVA) was performed on each variable at each data collection point to determine if there was a significant difference between the early and late arrivals. For all such analyses, an adjustment was made to compensate for the effects due to dosage group. It was found that the early and late arrivals differed significantly in several variables, including the following: daily urine volume, urine pH, urine total protein and leucine aminopeptides (LAP), fecal zinc, lung weight, intestine weight, and kidney and spleen weights.

Finally, because of the large interindividual variation of some variables, it was not possible to detect significant group differences even when the data were pooled for all 8 rats within each group. For each variable, a separate 1-way ANOVA was performed at each sacrifice to determine group differences at these individual time points. Also, for each variable a 2-way ANOVA was performed in which there was only one time interval, namely 0-24 months. For the 2-way ANOVAs, an adjustment was made to compensate for age effects. By the latter analysis, a maximum sample size of 68 rats per group was available and as a result, significant group differences often were detected for the study overall, whereas they could not be detected at the individual sacrifices. Additionally, the group mean differences were tested for significance at p values of both 0.05 and 0.10 using the Scheffe test. Using a larger, yet not unreasonable, p value was justified as the analyses were being used to test for differences between groups in which the sample sizes were small, the group means were often close, and the variances were often large.

As noted in the Materials and Methods section, Bartholomew's test for ordered means (Shirley, 1979) also was used to test for significant effects of Cd exposure on certain urine parameters and renal tissue changes. It was hoped that this test would be more sensitive than the ANOVA for detecting subtle changes due to Cd exposure.

FOOD AND WATER CONSUMPTION AND RAT WEIGHT GAIN

Food and water consumption and animal growth all relate to the health status of the animal. As expected, all three of these variables were affected by cadmium exposure. To a lesser degree, these variables also were affected by age, irrespective of Cd exposure.

As indicated in Figure 4-1, rat body weight varies inversely with the dose. From the second to the fifteenth month of the experiment, the Control animals averaged 50 grams heavier than animals in Group 4. This weight difference increased to 100 grams by the eighteenth week and to 150 grams by the end of the study.

Analysis of variance was performed on the rat weight data at each sacrifice and for the overall study, regardless of the individual sacrifices. At 3, 6, and 9 months, the Group 4 rats were significantly smaller than the Controls (at $p \leq 0.05$). Thereafter, because of decreasing sample size and large variance within each group, it was not possible to detect significant differences between the groups. However, the 2-way ANOVA for the whole study showed that overall, Group 4 rats were significantly smaller than Control rats ($p \leq 0.05$).

Figures 4-2 and 4-3 illustrate the group food and water consumption patterns, respectively. Group running mean values were calculated by averaging the weekly mean values for each group at consecutive four week intervals.

Food consumption varied inversely with dose. Analysis of variance was performed on the data at each sacrifice point and for the study as a whole. The results indicated that overall, Group 4 rats consumed significantly less food than did the Controls or Group 2. Also, Group 3 rats consumed significantly less than the Controls ($p \leq 0.05$).

Water consumption also varied inversely with dose. The highest dosed rats drank approximately half as much water as the Controls throughout most of the study. Analysis of variance of these data indicated that from 0 to 21 months, Group 4 rats were drinking significantly less water than all other groups and that after 21 months, they were drinking significantly less than the Controls ($p \leq 0.05$). For the study as a whole, Group 4 water consumption was significantly less than all other groups and Group 3 was significantly less than the Controls and Group 2 ($p \leq 0.05$). Additionally, age had a significant effect on water consumption in all groups. Consumption peaked around 3 months, declined until 7 months, and then remained stable until roughly 19 months, at

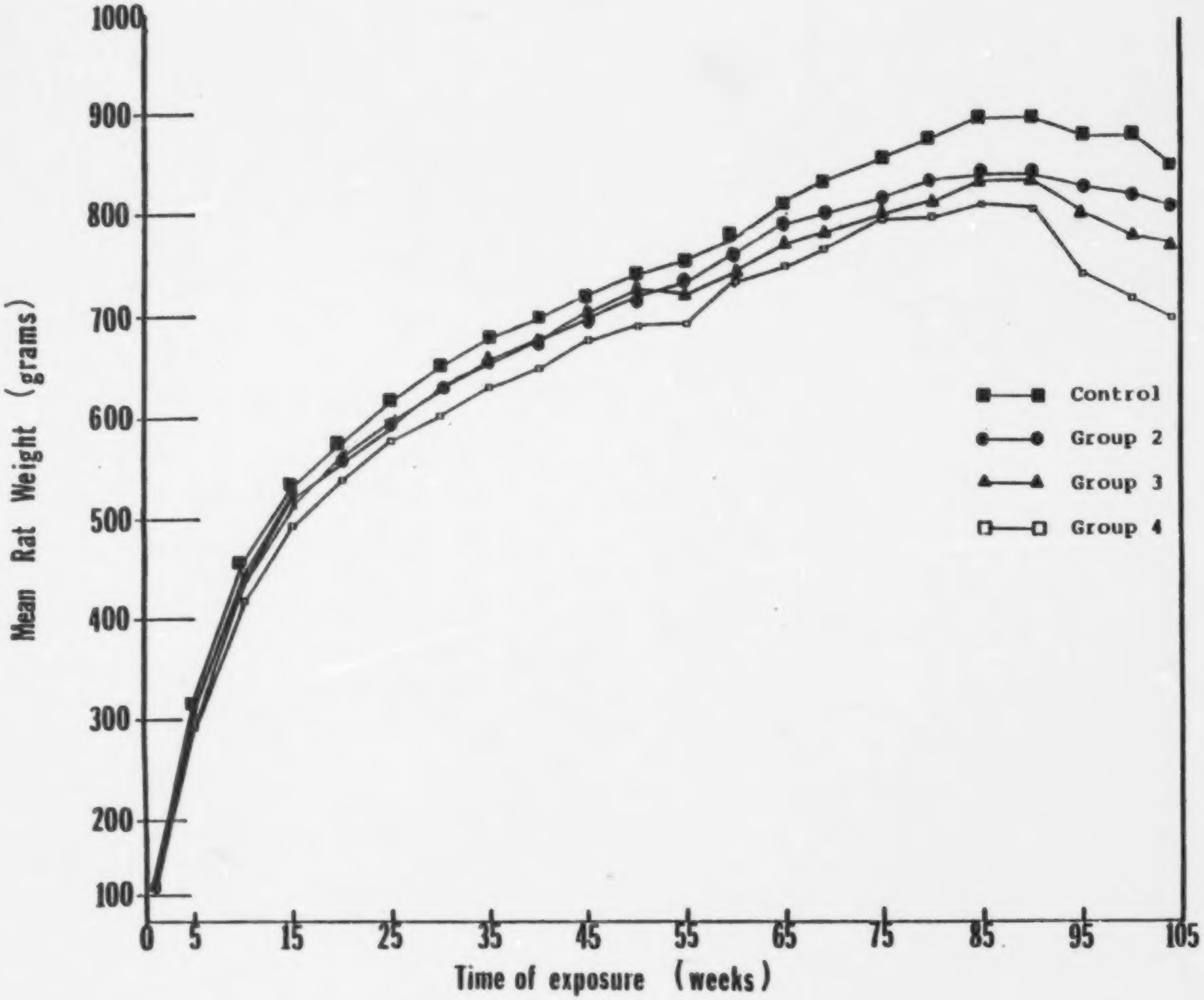


Figure 4-1. Mean Rat Body Weight (grams) as a Function of Age and Exposure to Cadmium

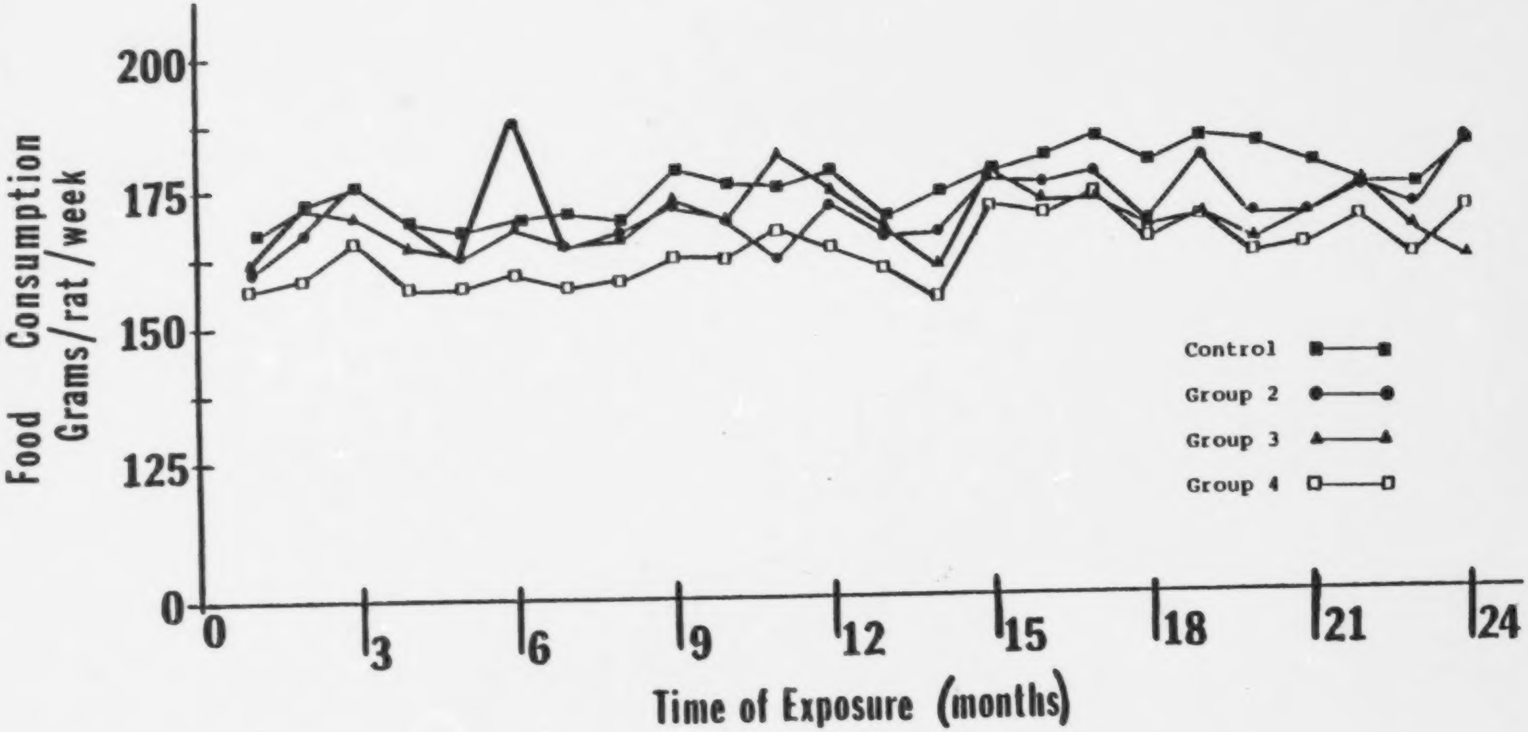


Figure 4-2. Running Mean of Weekly Food Consumption (grams of food/week/rat) as a Function of Time Exposure to Cadmium

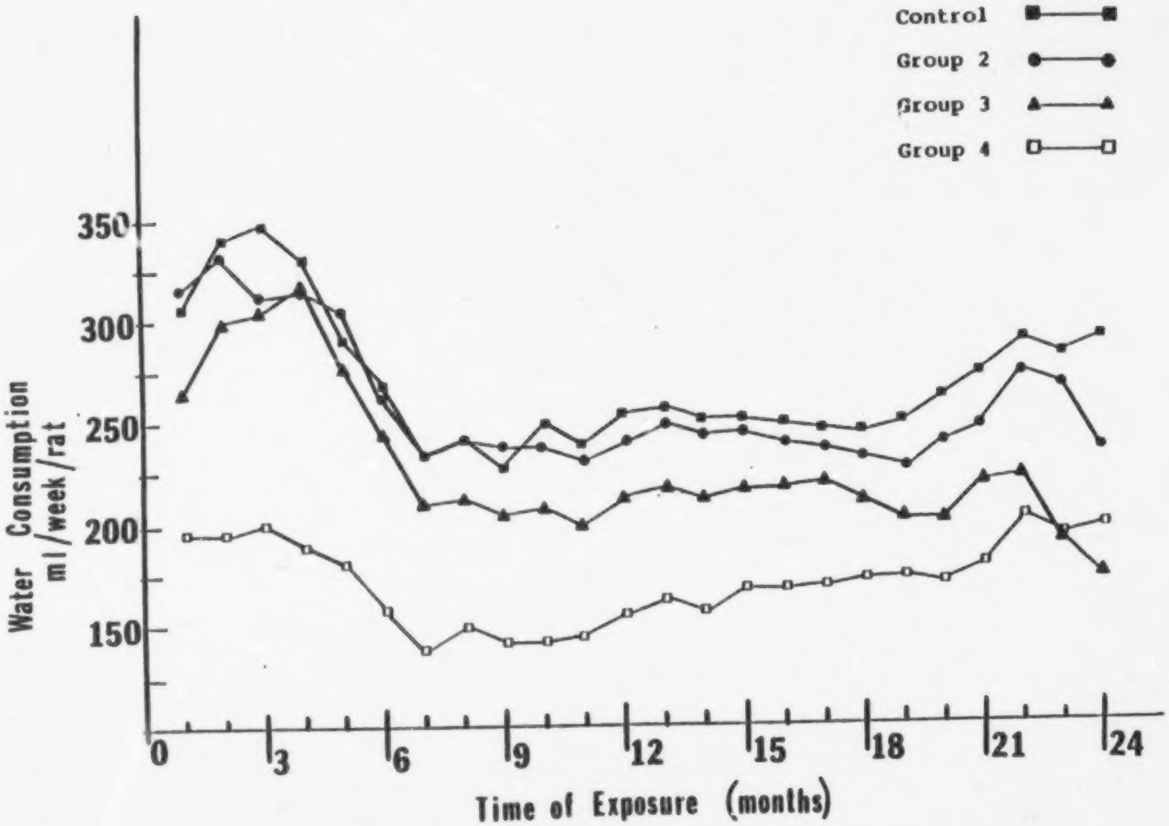


Figure 4-3. Running Mean of Weekly Water Consumption (ml of water/week/rat) as a Function of Time of Exposure to Cadmium

which point it increased again. After 21 months, there were 8 or fewer rats left per group, so that the significant reduction in water consumption seen in Groups 2 and 3 may have been related more to the health status of these few remaining animals rather than have been representative of the groups.

By influencing water consumption, the dosing regime and age also affected the amount of Cd actually ingested by the rats. As noted previously, the concentrations of Cd in the water of the test groups increased by multiples of 10 (i.e., 0.5, 5.0 and 50 μg/ml). However, when the dosages were recalculated as μg Cd/day/kg rat weight, based on water consumption, it was seen that the actual doses to the rats changed with time and did not stabilize until the sixth month (Fig. 4-4). The formula used for this conversion was as follows: For each test group, at any one week, μg Cd/day/kg rat weight =

$$\frac{(\mu\text{g/Cd/ml water})(\text{group mean water consumption as ml/wk/rat})}{(14\text{ days/wk})(\text{group mean rat weight in kg})}$$

By the sixth month, animals from all three exposure groups were consuming about 25 percent of the dose ingested at the beginning of the study. From the sixth month to the termination of the study, the actual dosages to the rats were not multiples of 10, but increased by a factor of 8, between the 0.5 and 5.0 μg/ml groups, and by a factor of only 7.8 between the 5.0 and 50 μg/ml groups.

Finally, the data presented in Figure 4-4 were recalculated to show the accumulated exposures, as mg Cd/kg rat weight. To accomplish this, the area under each curve was determined from the zero time up to each sacrifice point. The data are as follows:

	Accumulated Exposure (mg Cd/kg rat weight) (months)							
	3	6	9	12	15	18	21	24
Group 2:	6.47	9.24	11.97	14.21	16.38	17.92	19.74	21.63
Group 3:	57.75	83.23	107.17	126.14	146.23	159.88	173.74	186.62
Group 4:	401.87	566.51	739.06	885.57	1041.90	1160.00	1290.50	1432.10

Thus, animals sacrificed at the end of the study were exposed to a total of 21.63, 186.62 and 1,432.1 mg Cd/kg body weight in Groups 2, 3 and 4, respectively.

HEMATOCRIT

Figure 4-5 illustrates the group mean hematocrit levels as a function of time of exposure to Cd. Overall, there was no significant difference in

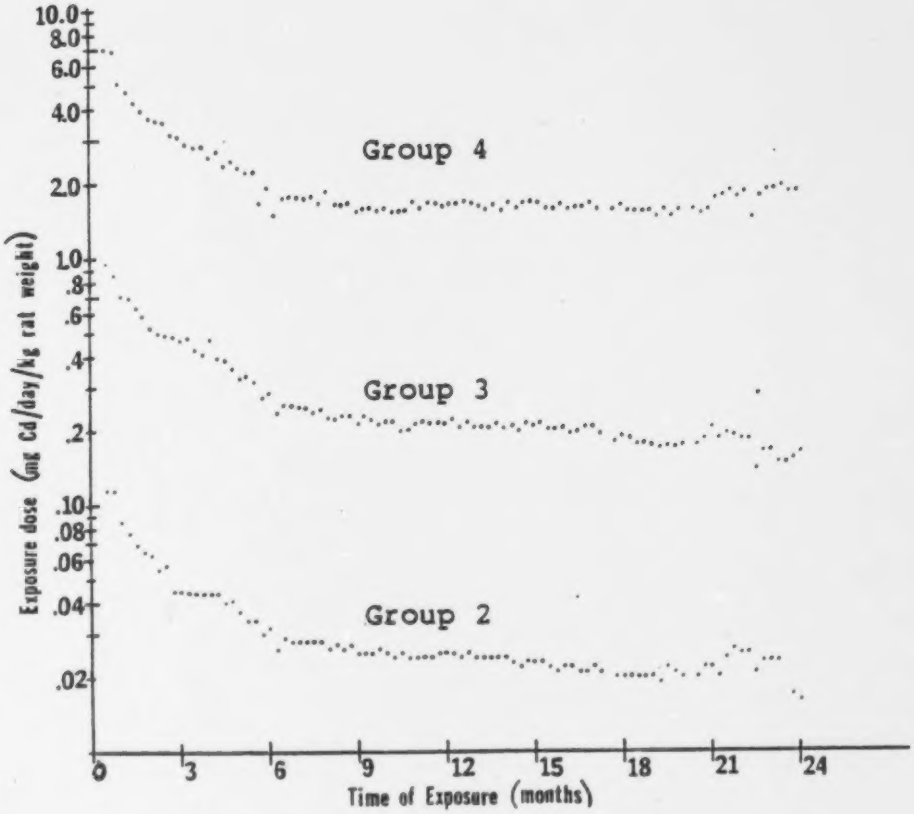


Figure 4-4. Cadmium Exposure (mg Cd/day/kg rat weight) as a Function of Age, Rat Weight, and Water Consumption

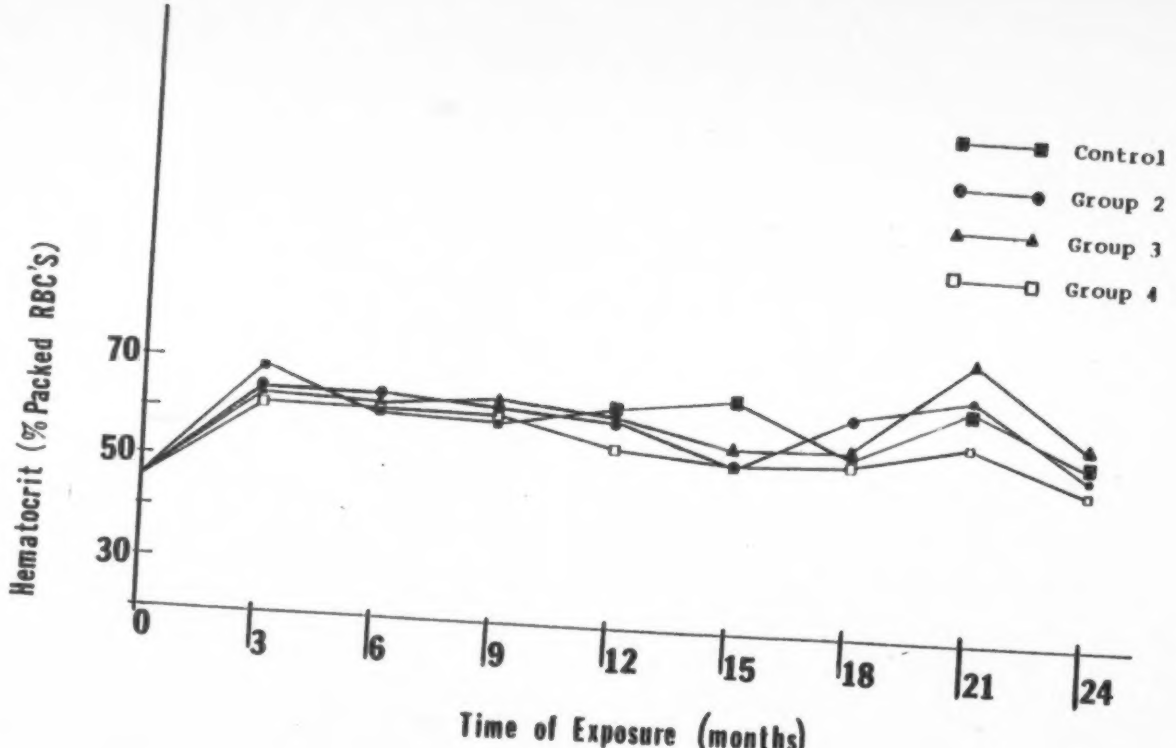


Figure 4-5. Hematocrit (as percent packed red blood cells, RBC's) as a Function of Age and Exposure to Cadmium

hematocrit levels between the early and late arrivals; however, there was a significant difference associated with age and level of Cd exposure. Looking at the whole experimental period of 24 months, irrespective of individual sacrifice points, the hematocrit levels of Group 4 were significantly lower than those of the Control or Group 3 at $p \leq 0.05$ (see Appendix Table A-1). Over the 24 months, there was a negative correlation between age and hematocrit for all groups; however, this correlation was significant only for the highest dose group, Group 4.

To examine the relationship between the concentration of Cd in the kidney and hematocrit, data from 0-15 months were used. This time period was selected since renal Cd levels for all experimental groups peaked at 15 months (see Fig. 4-7) and then decreased significantly thereafter. In examining the data by group, a significant negative correlation was noted between renal Cd and hematocrit for Groups 3 and 4. In examining all the data for this time period, irrespective of group, a significant negative correlation was seen between renal Cd and hematocrit. Since renal Cd increased with age during this time period, the relationship between age and hematocrit was assessed. For 0-15 months, there was a negative correlation between age and hematocrit for all groups, but it was significant only in Groups 3 and 4. Interestingly, when the data were aggregated for this period and analyzed irrespective of group, the correlation was negative but not significant. In all correlations involving hematocrit, a p value of 0.05 or less was used to test for significance.

FECAL METALS

Figures 4-6A, -B, and -C illustrate the concentrations of Cd, Zn and Cu, respectively, in the feces. After adjusting for group effects, it was found that the early and late arrivals differed significantly only in their levels of fecal Zn. The early arrivals excreted more Zn than did the late arrivals. For purposes of graphing the data, the fecal Zn values for both early and late rats were pooled for each test group at each time point. On the other hand, when analyzing the different dosage groups for statistically significant differences, the early and late arrivals were treated separately.

As expected, the excretion of Cd was dose dependent. Throughout the entire experiment, the levels of fecal Cd from Group 4 were significantly higher than those from all other groups (see Appendix Table A-2). For the most part, Group 3 levels also were significantly higher than the Control and Group 2 levels. Group 2 levels were not found to be significantly different from Control values.

There were no significant differences between the groups for fecal copper. Also, after adjusting for differences between early and late arrivals, there was no significant group effect on fecal Zn.

Initially, it was planned that by retaining the rats in metabolic cages and measuring the exact water consumption, and thus the total daily Cd intake, plus the total daily elimination of Cd via the feces, it would be possible to estimate the percentage of the dose that was absorbed by the body. Since it was assumed that excretion of Cd via the urine would be insignificant until there was extensive kidney damage, this approach should have yielded a

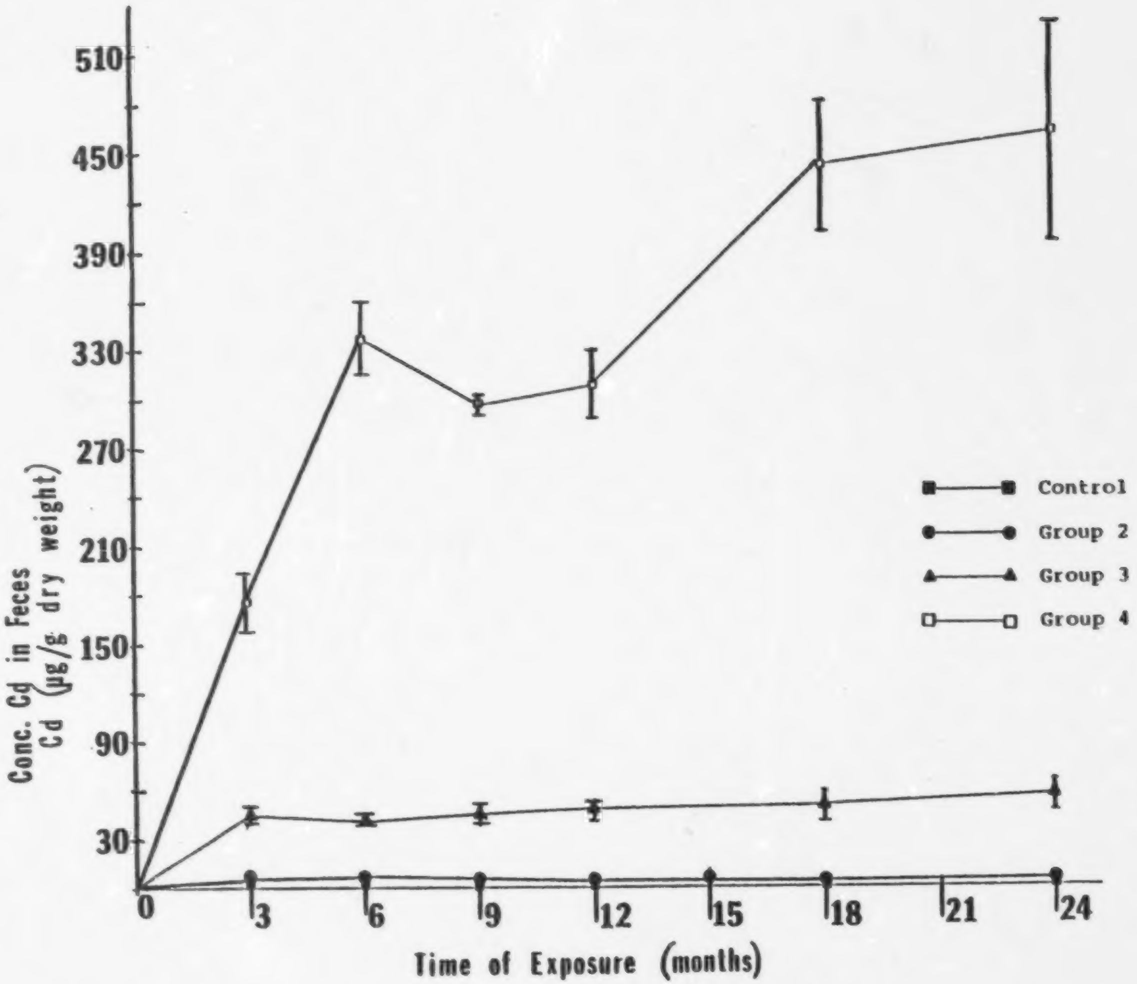


Figure 4-6A. Fecal Cadmium (µg Cd/gram dried feces, mean ± S.E.) as a Function of Exposure to Cadmium

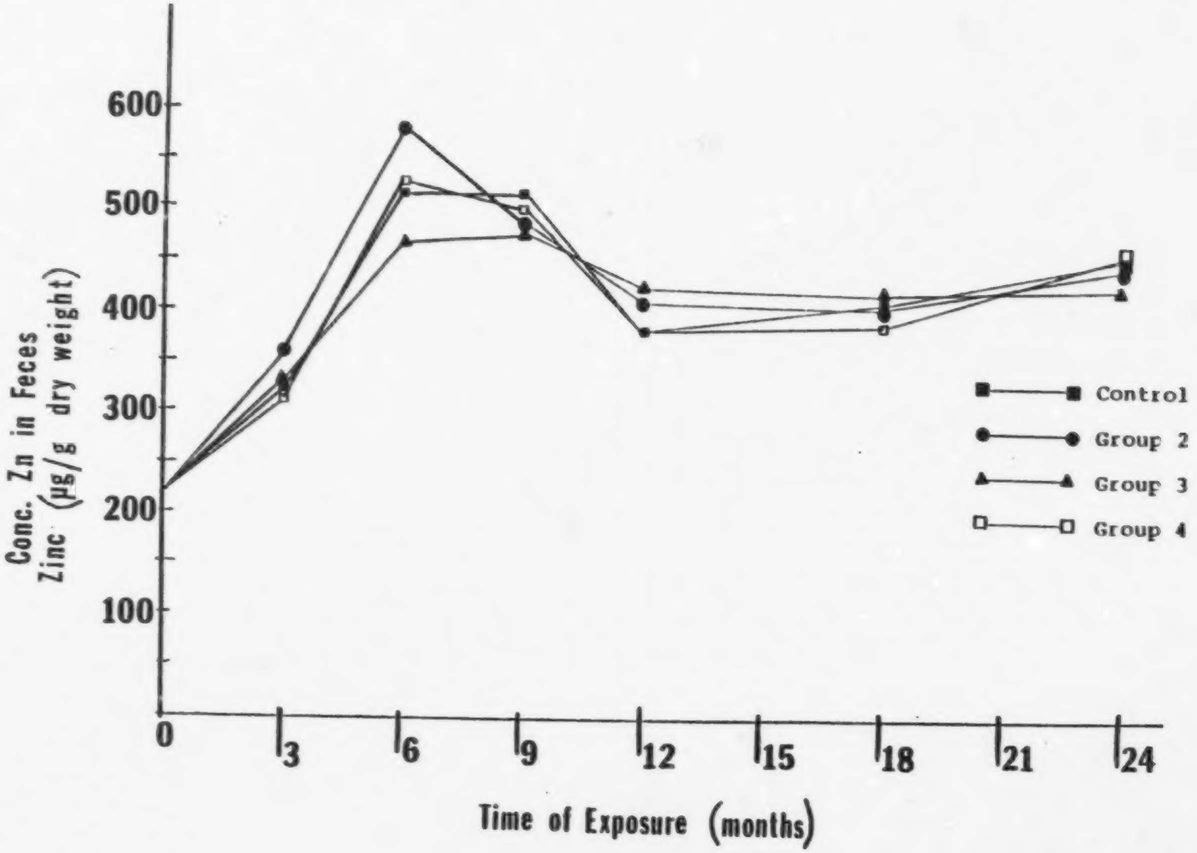


Figure 4-6B. Fecal Zinc (µg Zn/gram dried feces) as a Function of Time of Exposure to Cadmium

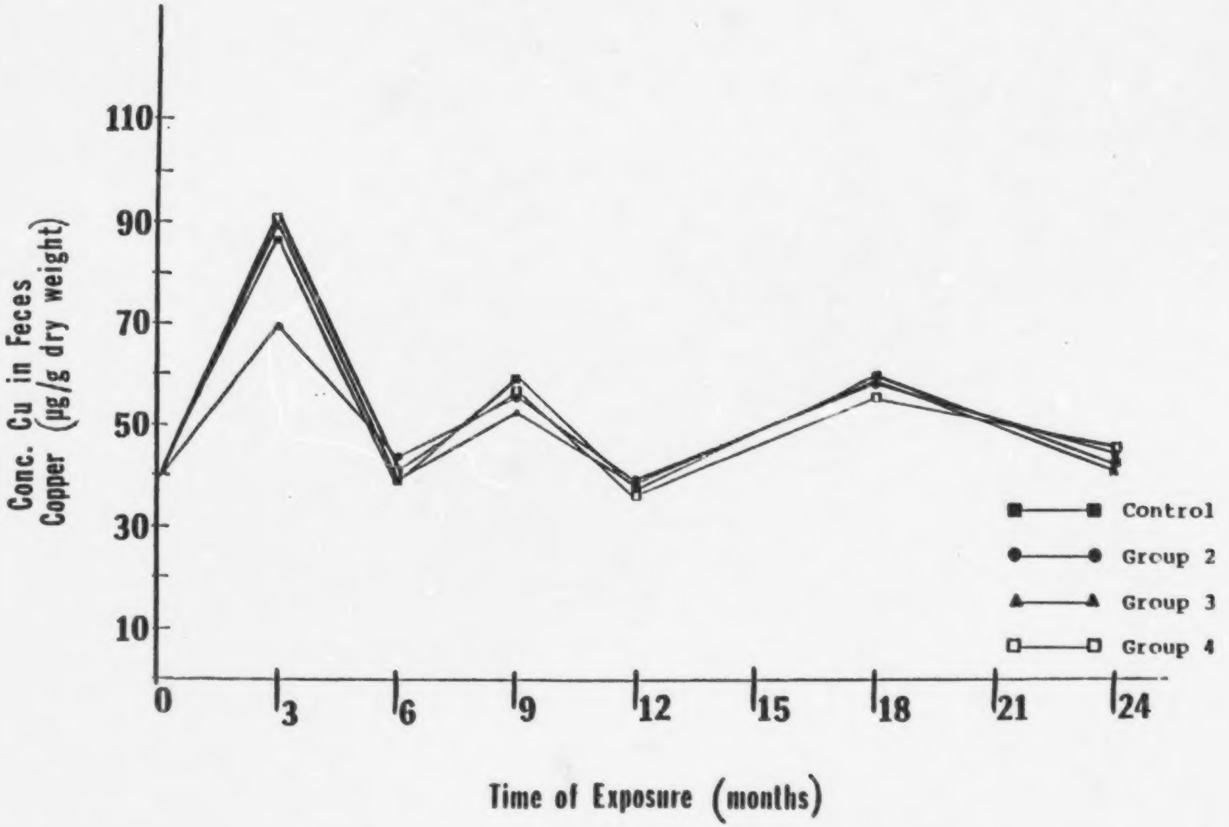


Figure 4-6C. Fecal Copper (µg Cu/gram dried feces) as a Function of Time of Exposure to Cadmium

reasonable estimate over most, if not all, of the study. Unfortunately, certain problems were encountered with the use of the metabolic cages and, as a result, these calculations could not be performed. The rats, normally housed in cages quite different from the metabolic cages, did not like being in the latter even for brief periods. As a result, many of the animals drank less water and ate less food than normal and, therefore, excreted amounts of feces which were not considered to be representative of normal daily outputs. Even though the rats were acclimated for 48 hours in the metabolic cages prior to collection of fecal samples, many simply did not perform normally. This problem was discussed with veterinarians from the Department of Comparative Medicine, with researchers from the National Institutes of Health, and with technical representatives of Nalge Company, a manufacturer of metabolic cages. This appears to be a fairly common problem, particularly with old rats. The only reasonable solution was to raise the animals in the metabolic cages. Because of the experimental design, numbers of rats involved, and availability of equipment, this course of action was impossible. Thus, only the concentrations of the metals in the feces and not the total amount of metals excreted via this route have been calculated.

ACCUMULATION OF CADMIUM, ZINC AND COPPER IN THE KIDNEY

Renal Cadmium

Figure 4-7 shows the concentration of Cd (μg/g, dry weight) in the intact renal cortex for the three test groups. Each point represents the mean Cd concentration ± standard error (S.E.) for the kidneys of 6-8 rats. It is seen that Cd accumulation is linear and dose dependent, at least to the 15 month point. At this peak, the corresponding group mean concentrations on a wet weight basis (μg Cd/gram wet weight) are 2.56, 11.54 and 92.0 for Groups 2, 3 and 4, respectively. Comparison of the slopes of the curves from 0 to 15 months shows that the rate of Cd accumulation in the cortex increases by a factor of 5 between Groups 2 and 3 as compared to a factor of 8 in the actual exposure dose to the rats (Fig. 4-4). The cortical Cd accumulation rate increased by a factor of 8.6 between Groups 3 and 4, as compared to an increase in the actual dose to the rats of 7.8.

Analysis of variance of this cortical Cd data indicated that at each sacrifice, and overall, Group 4 had Cd levels that were significantly higher than those in any of the other groups (Appendix Table A-4). Additionally, at 15 and 18 months, and overall, Group 3 levels were significantly higher than Control levels. Throughout the study, a significant difference could not be detected between Control and Group 2.

Overall, there was no significant difference in cortical Cd levels between the early and late arriving rats (Appendix Table A-3). There appeared to be a significant difference only at 0, 6 and 21 months; however, this did not alter the results of the ANOVA (Appendix Table A-4) used to test for significant differences between the groups.

Figure 4-8 shows the concentration of Cd (μg/g dry weight) in the renal cortical tubules. Accumulation of Cd in the tubules showed a similar pattern to that of the intact cortex. Thus, accumulation was both dose and age

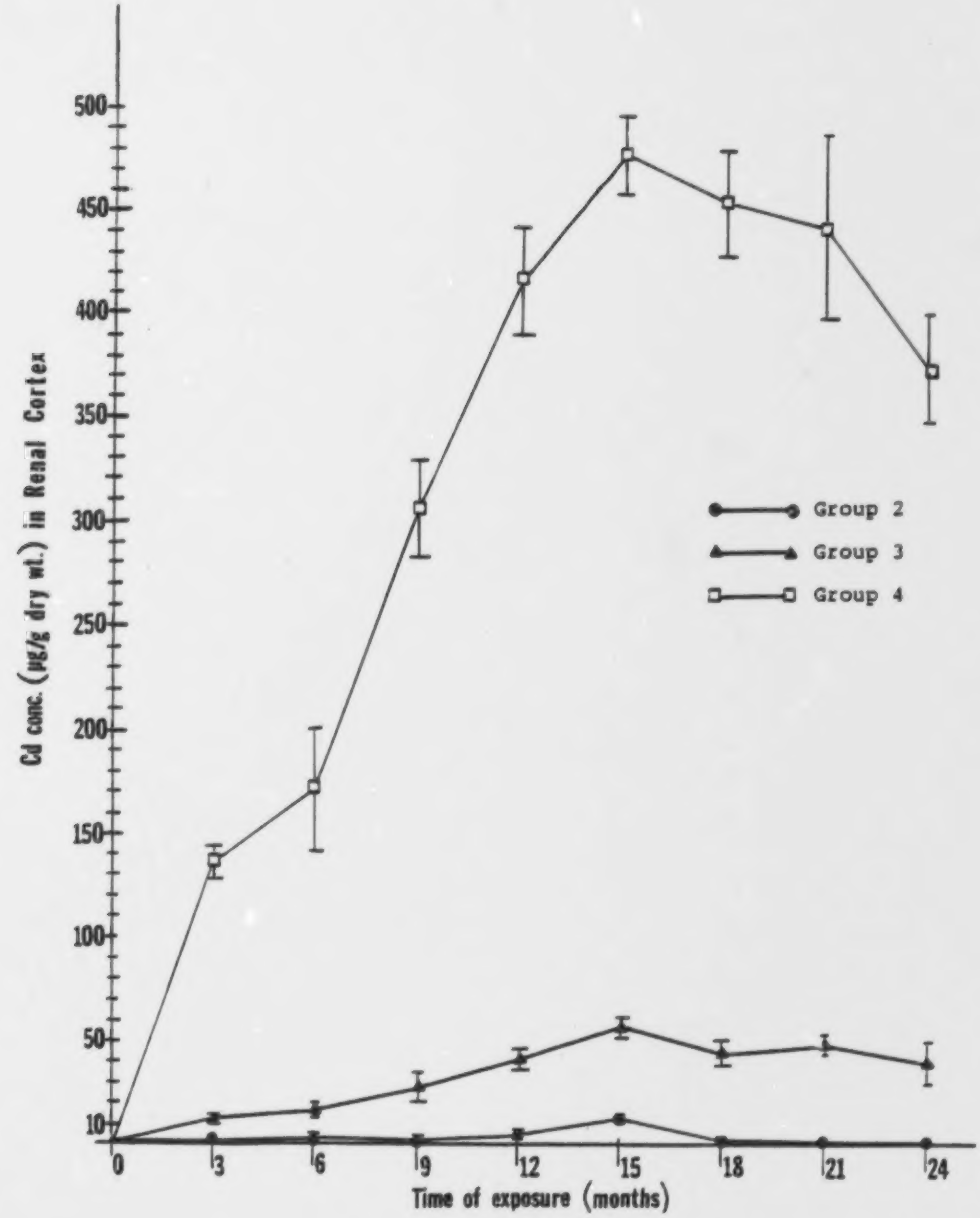


Figure 4-7. Concentration of Cadmium in the Intact Renal Cortex (µg Cd/g dried cortex) as a Function of Exposure to Cadmium

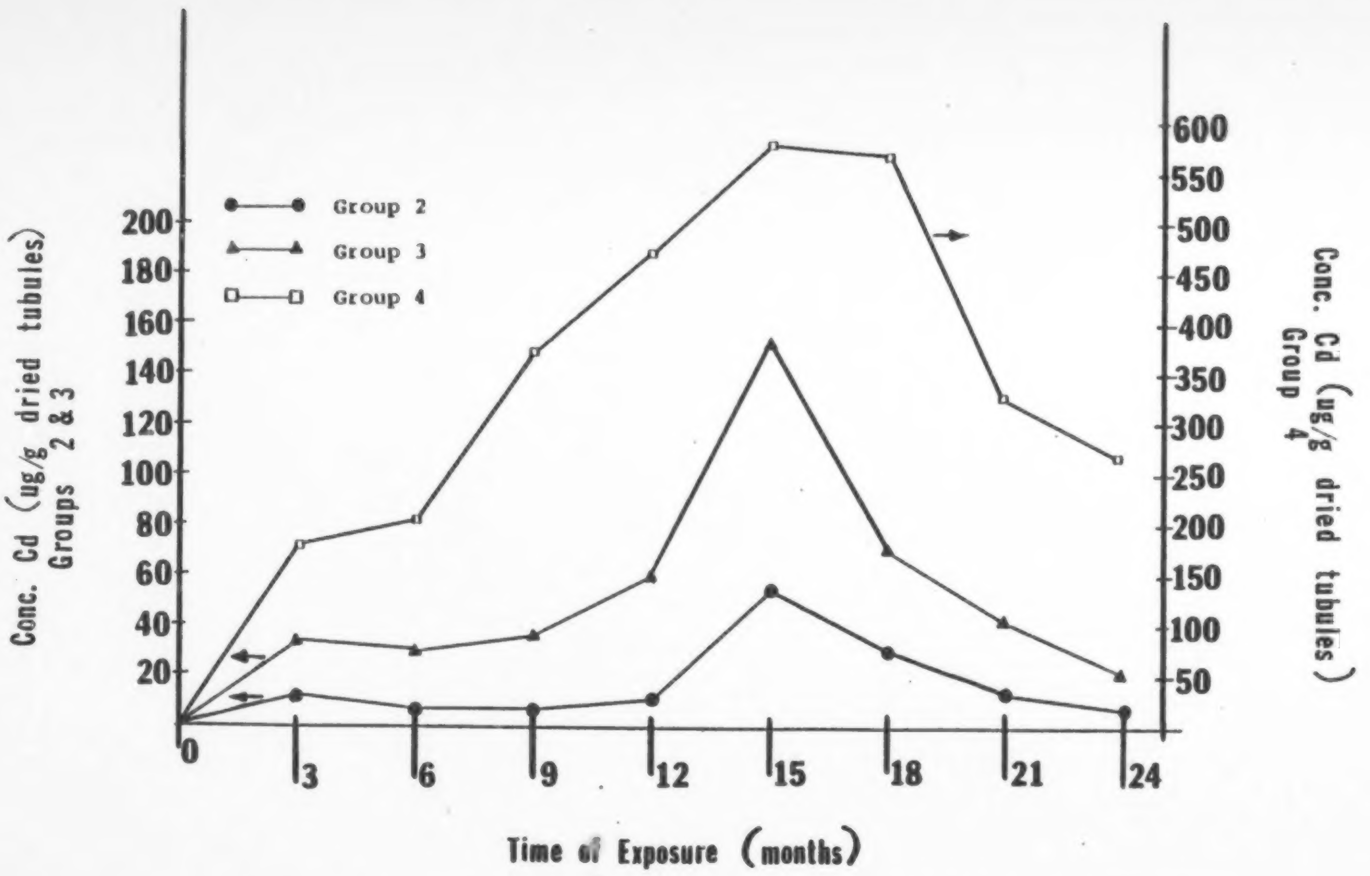


Figure 4-8. Concentration of Cadmium in the Renal Cortical Tubules as a Function of Exposure to Cadmium

dependent and increased with time of exposure up to 15 months. After 15 months, the concentration in all groups decreased.

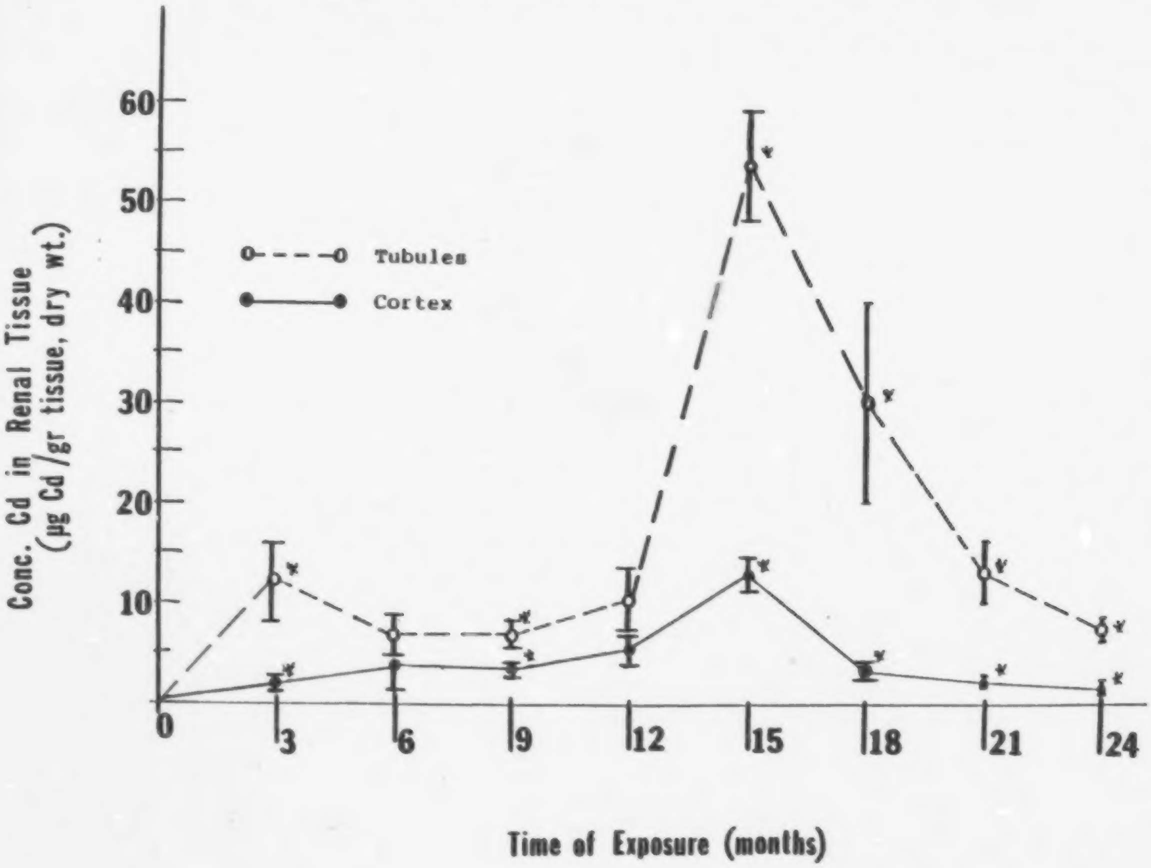
There was no significant difference in tubular Cd levels between the early and late arriving rats. Analysis of variance indicated that throughout the study, Group 4 had significantly higher concentrations of Cd than all other groups (Appendix Table A-5). At 15 months, Groups 2 and 3 were significantly different from each other and Group 3 was significantly higher than Control. Overall, Group 3 had significantly higher concentrations than did the Controls. Similar to the situation with the intact cortex, cortical tubular Cd levels were not significantly higher in Group 2 as compared to Controls.

The relationship between the concentration of Cd in the intact cortex and the concentration of Cd in the cortical tubules for the three dosage groups is illustrated in Figure 4-9, A-C. The data are calculated as μg Cd per gram of dried tissue and each point represents the mean ± S.E. for the tissues of 6 to 8 rats. Individual paired t-tests were performed on the data from each rat in order to compare the concentrations of tubular and cortical Cd. Up to 18 months, with few exceptions, the tubules consistently had significantly higher concentrations of Cd than did the cortices ($p \leq 0.05$). This effect was seen in all three exposure groups. Additionally, in the Controls, the levels of Cd were significantly greater in the tubules than the cortex for 9, 21, and 24 months. It should be noted that statistical analysis of the Control group data was weakened since many of the animals had Cd concentrations below the detection limits of the atomic absorption spectrophotometer. The data indicate, therefore, that Cd accumulates to a greater extent in the cortical tubules relative to the whole cortex.

As shown in Figure 4-9A, cortical tubular Cd concentrations in Group 2 remained significantly higher than cortical Cd concentrations for the entire study. In contrast, in Groups 3 and 4, the cortical tubular Cd concentrations were significantly higher, relative to the cortical Cd concentrations up to 18 months (Fig. 4-9B and -C) and thereafter were lower than the cortical Cd levels. The cortical tubular Cd concentrations were significantly lower than the cortical levels at 21 months for both Groups 3 and 4 and at 24 months for Group 4.

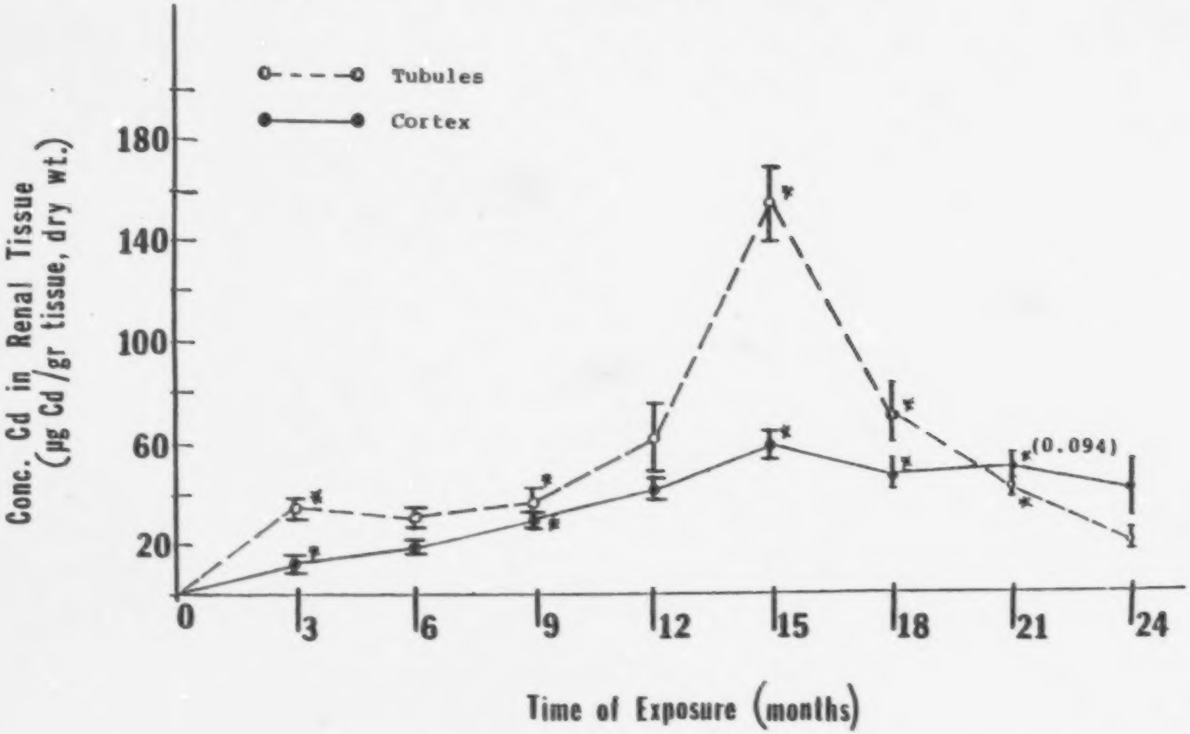
The shapes of the curves in Figure 4-9, A-C suggest that the pattern of Cd accumulation in the cortical tubules differs somewhat from that in the intact cortex. In Group 4, the Cd concentration in both the cortex and the cortical tubules increased linearly with time of exposure up to 15 months. Between 15 and 18 months, the levels of Cd decreased slightly in both the cortex and the cortical tubules; however, the tubules still maintained a higher concentration of metal relative to the cortex. After 18 months, the cortical Cd concentrations continued to decline at a gradual rate, decreasing by approximately 21 percent between the peak value at 15 months and the study termination at 24 months. In contrast, the cortical tubule Cd concentration decreased quite rapidly after 18 months, declining an estimated 47 percent between the 15 and 24 month sacrifices.

Unlike the situation in Group 4, the increase of cortical tubular Cd in the two lower exposure groups was linear only up to the 12 month point, while the



*Significant difference between tubules and cortex at $p \leq 0.05$.

Figure 4-9A. Concentration of Cadmium in the Intact Renal Cortex and Cortical Tubules as a Function of Exposure in Group 2 (0.5 µg Cd/ml)



*Significant difference between tubules and cortex at $p \leq 0.05$.
*(x)Significant difference at $p = x$.

Figure 4-9B. Concentration of Cadmium in the Intact Renal Cortex and Cortical Tubules as a Function of Exposure in Group 3 (5.0 µg Cd/ml)

*Significant difference between tubules and cortex, p ≤ 0.05.
*(x)Significant difference at p = x.

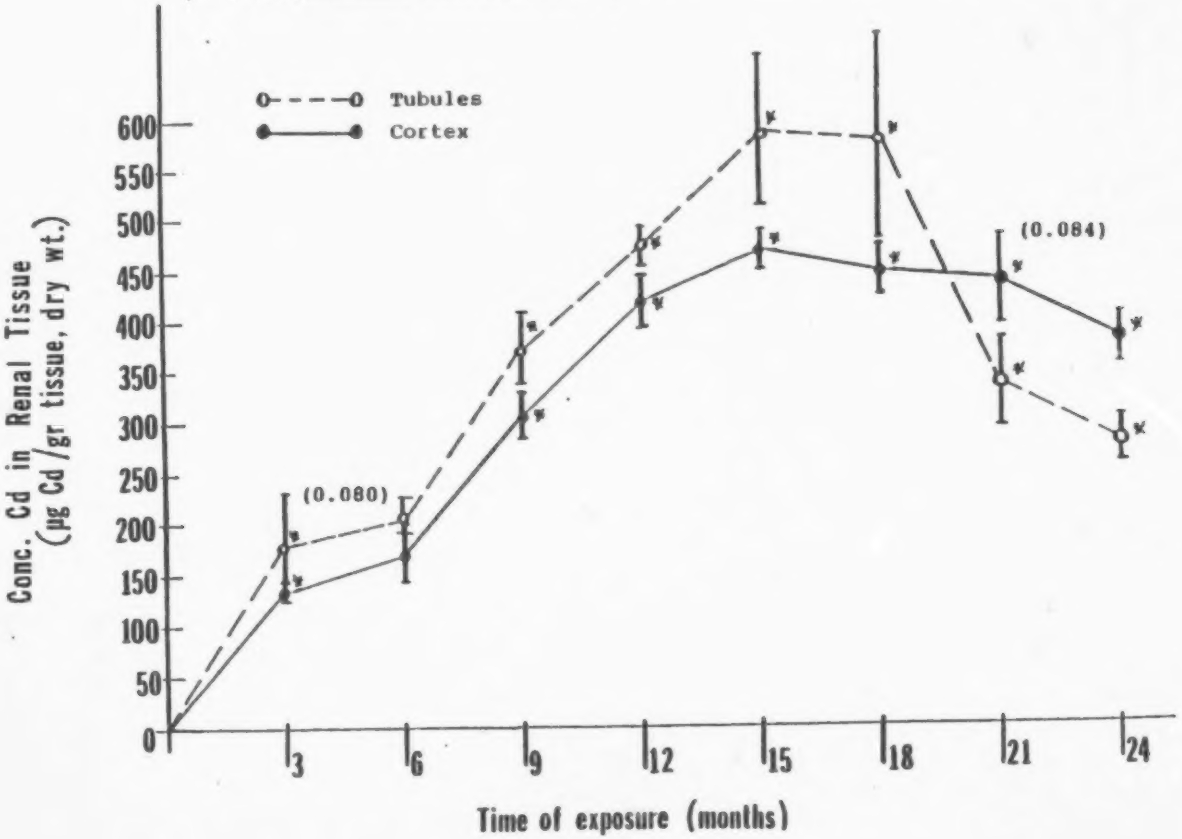


Figure 4-9C. Concentration of Cadmium in the Intact Renal Cortex and Cortical Tubules as a Function of Exposure in Group 4 (50 µg Cd/ml)

increase in cortical Cd was linear up to the 15 month sacrifice (Fig. 4-9A and -B). Between 12 and 15 months, the concentration of Cd in the cortical tubules increased quite rapidly with respect to cortical levels. After peaking at 15 months, the levels in both the cortex and cortical tubules decreased. As seen in the highest dose group, the decline of Cd concentration was much more rapid in the tubules than in the whole cortex for Group 3. Thus, between 15 and 24 months, mean cortical Cd in Group 3 decreased by 30.6 percent while mean cortical tubular Cd decreased by 86.5 percent. In Group 2, mean cortical Cd decreased by 87.4 percent, while mean cortical tubular Cd decreased by 86.3 percent. The data seem to indicate that the rate of decrease in renal Cd levels was inversely related to dose.

It was noted that the physical integrity of the renal tubule preparations appeared to change with the age of the rats, irrespective of the level of Cd exposure. Up to the 21 month sacrifice, almost all of the preparations were considered to be good in that they consisted primarily of short, intact tubules, with few glomeruli and little cellular debris. In contrast, many preparations made during the 21 and 24 month sacrifices had relatively few intact tubules, and greater proportions of cellular debris and glomeruli. Also, at 24 months, the majority of preparations contained many amorphorus masses of varying size but generally circular shape. These structures, which were never seen prior to the last sacrifice, were unlike any renal tissue normally found in these preparations.

In order to estimate the relative rate of accumulation of Cd in the cortical tubules versus the intact cortex, the linear regression of Cd concentration against time of exposure was calculated for each experimental group for both the cortical tubules and the intact cortices. Only the linear portion of each curve was used. The ratio of the slopes of these two lines was then calculated by dividing the slope of the tubule accumulation curve by the slope of the cortical accumulation curve. For the lower dosage groups, the ratio was calculated up to 12 months of exposure and showed values of 1.4 and 1.3 for Groups 2 and 3, respectively. For the high dose group, this ratio was calculated for data up to 15 months and had a value of 1.2. Thus, in the linear portion of the accumulation curves, the rate of Cd accumulation in the cortical tubules relative to the intact cortex was dose dependent and decreased with increasing dose.

Renal Copper and Zinc

The effects of cadmium exposure on the zinc and copper concentrations in the cortical tubules and whole cortex were examined. The data for copper and zinc are presented in Figures 4-10A, -B and 4-11A, -B, respectively. All data are presented as µg of metal per gram of dry tissue and each point represents the mean value for tissues of 6 to 8 rats.

Overall there was no significant difference between the early and late arriving rats in their levels of either cortical or tubular zinc. As indicated in Appendix Table A-3, there was a significant difference between the early and late rats only at 0 to 6 months for cortical zinc and only at 0, 15, 21 months for tubular zinc. Thus, throughout the study, there was no consistent pattern of difference in tissue zinc levels between the early and late arriving rats.

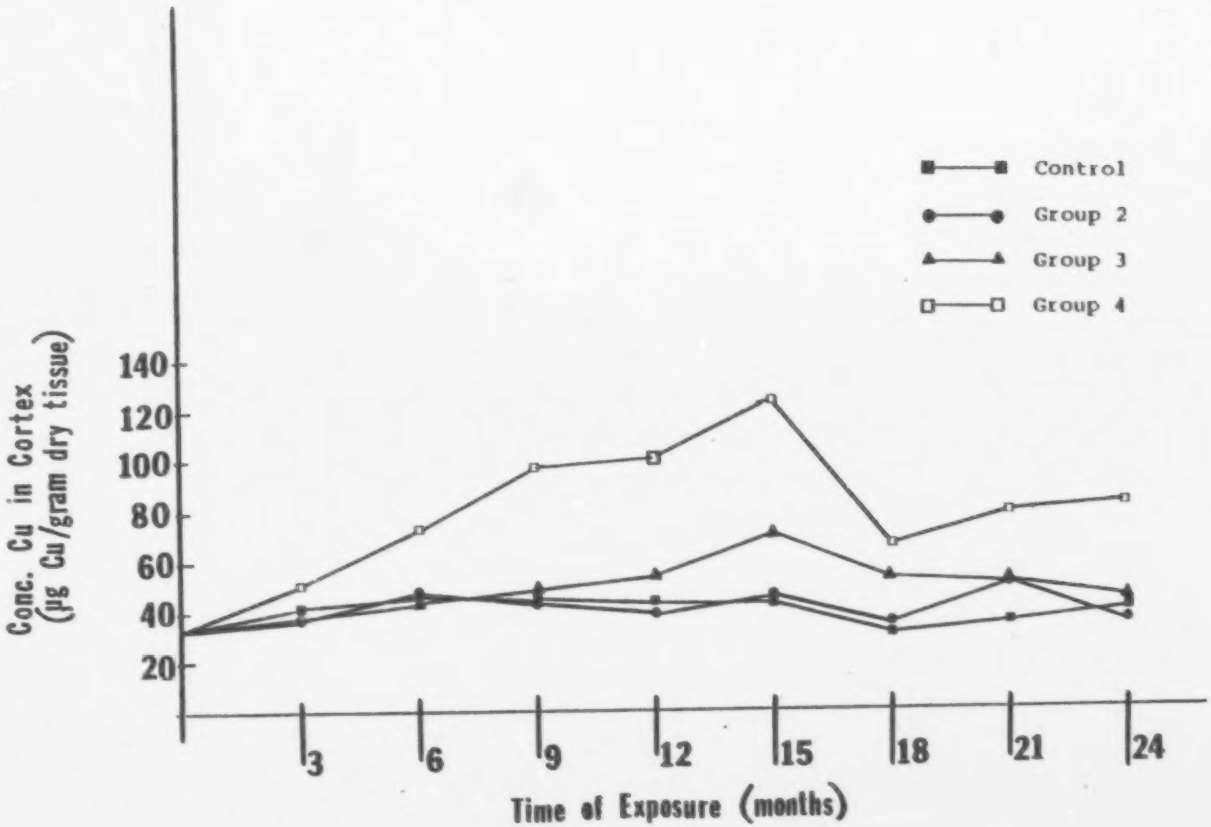


Figure 4-10A. Concentration of Copper in the Intact Renal Cortex as a Function of Age and Exposure to Cadmium

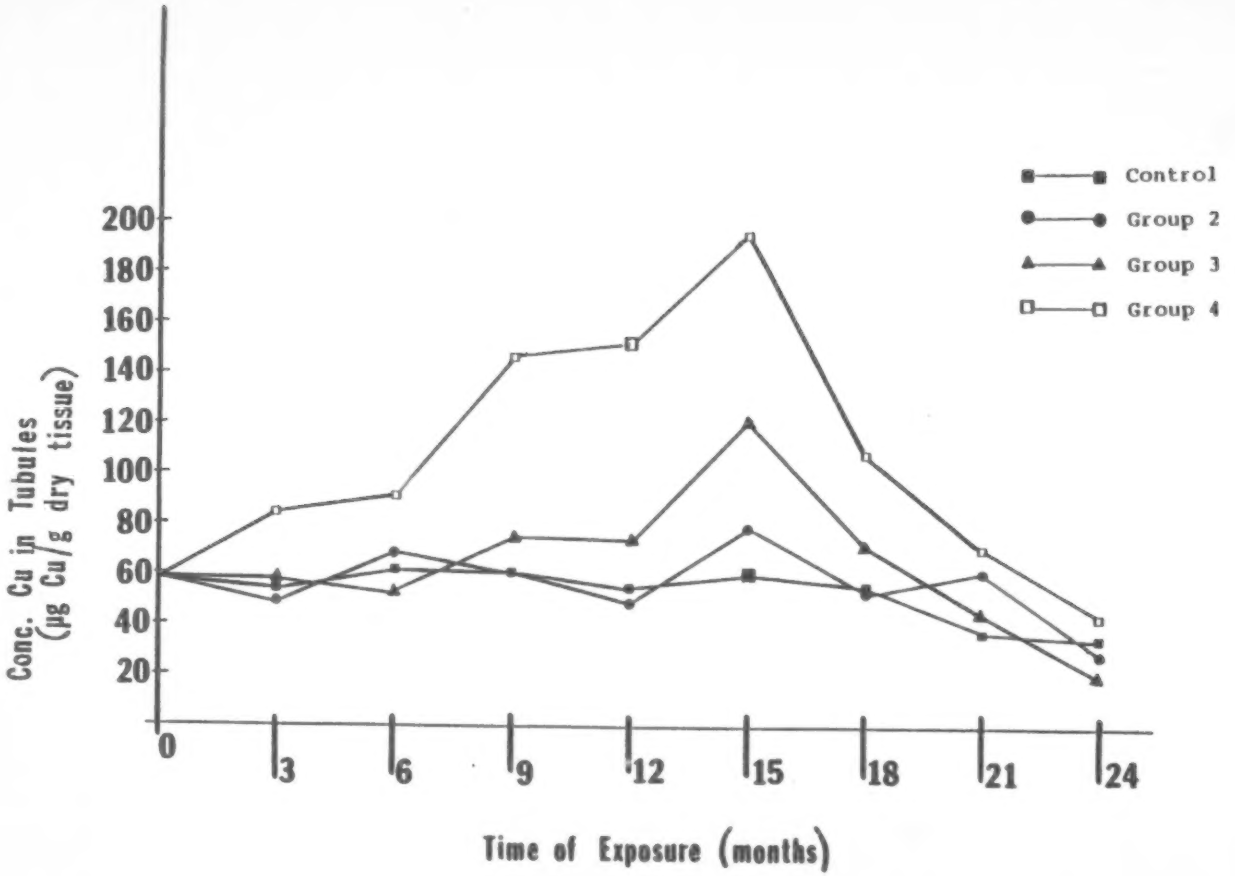


Figure 4-10B. Concentration of Copper in the Renal Cortical Tubules as a Function of Age and Exposure to Cadmium

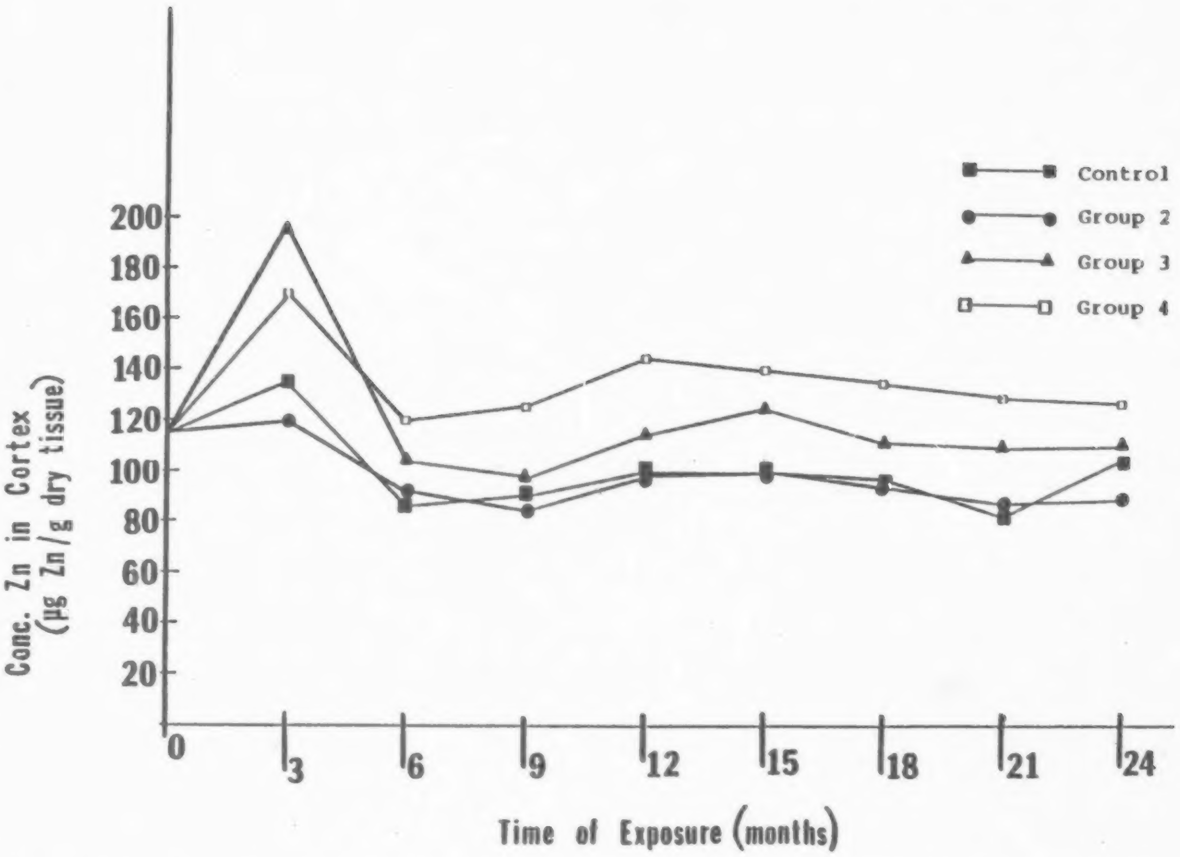


Figure 4-11A. Concentation of Zinc in the Intact Renal Cortex as a Function of Age and Exposure to Cadmium

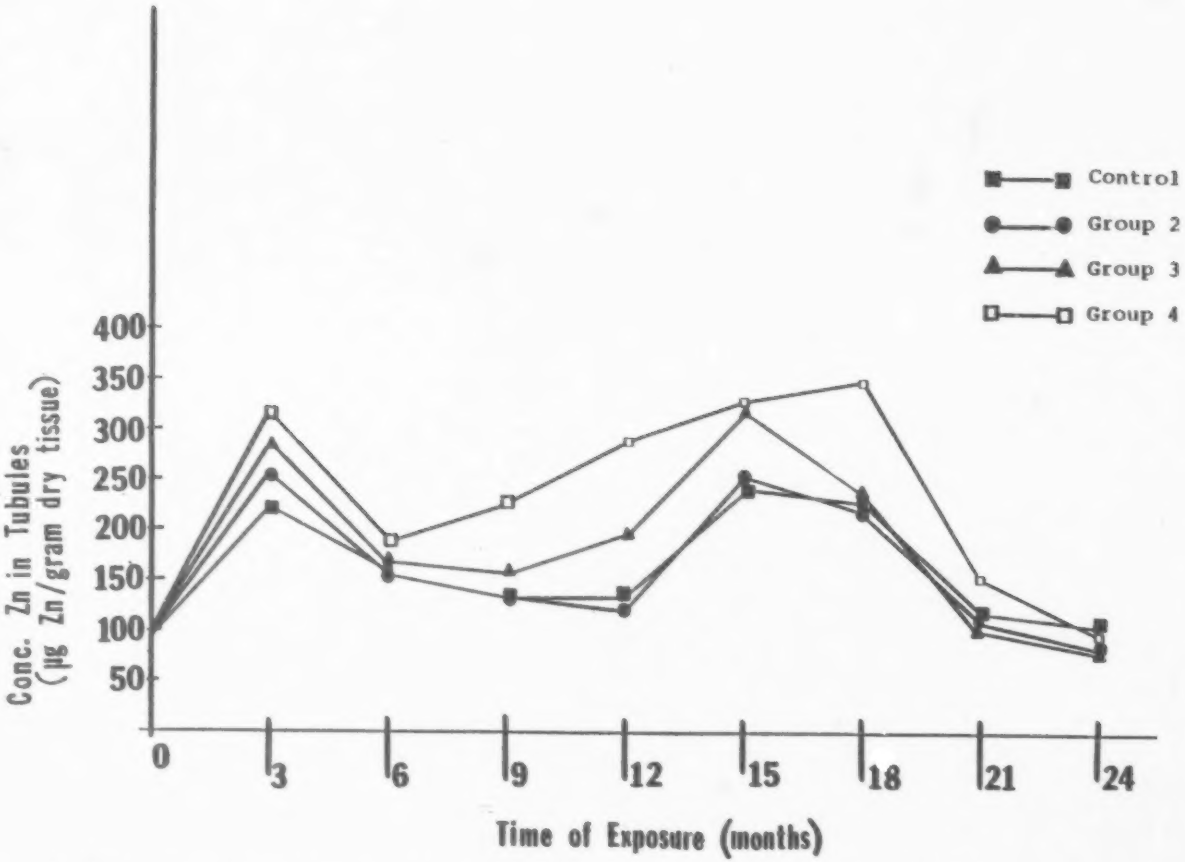


Figure 4-11B. Concentration of Zinc in the Renal Cortical Tubules as a Function of Age and Exposure to Cadmium

Further, the significant differences found at these isolated time points were small enough to be washed out when the data from the entire study were treated as a whole, irrespective of individual sacrifice points.

There appeared to be a significant difference between early and late arriving rats in their levels of cortical copper (Appendix Table A-3). This difference was apparent at the zero time point and then was not evident again until 18-24 months. Overall, for the two years, the two sets of animals differed significantly in their levels of cortical copper. A somewhat similar situation was seen in the cortical tubular copper levels. The early and late rats differed significantly in tubular copper only at 0, 18, 21 months; however, overall, there was no significant difference.

In order to present the data in the most accurate, yet least confusing manner, mean values for renal zinc and copper have been calculated and plotted for each dosage group (Figs. 4-10A, -B and 4-11A, -B) by treating the early and late sets of rats as one homogeneous group. The results of the ANOVA used to identify significant differences between exposure groups were checked and found not to be altered by the isolated time points at which the early and late arriving rats differed.

There was a clear, dose-response relationship between Cd exposure and the levels of both Zn and Cu in the intact cortex and cortical tubules. As shown in Figures 4-10 and 4-11, the 0.5 µg/ml dose (Group 2) had little effect, while the 50 µg/ml dose (Group 4) had the greatest effect on the elevation of both copper and zinc.

The results of 1-way ANOVAS performed at each sacrifice point to determine which dosage groups differed significantly from each other in renal Cu and Zn are presented in Appendix Tables A-6 through A-9. When the results for cortical Cu were summarized, Group 4 was significantly higher than all other groups at 6, 9, 12, 15, 24 months and for the overall study as a whole. There was no significant difference between any group at 3 or 21 months, and at 18 months, Group 4 was significantly higher than just the Control group. As expected, cortical tubular Cu showed a similar pattern. Group 4 was significantly higher than all other groups at 9, 12 months and overall for the study as a whole. Additionally, at 15 and 21 months, Group 4 was significantly higher than the Controls. At 6 and 24 months, Group 3 had the lowest mean values, so that it was significantly lower than Group 4 at these two time points. A significant difference in cortical and cortical tubular Cu levels could not be detected between the Control and Groups 2 or 3.

In cortical Zn concentrations for the overall study, both Groups 3 and 4 were significantly higher than Control and Group 2; however, Group 3 was not significantly different from Group 4, nor was Group 2 significantly different from the Controls. At 9, 18 and 21 months, Group 4 was significantly higher than all other groups.

As shown by the graphs (Fig. 4-11A, -B) and the statistical analysis, the pattern of accumulation of Zn in the intact cortex differed from that of Zn in the cortical tubules for all groups. This difference was most striking from 6 to 24 months, when cortical Zn levels appeared unaffected by age, while cortical tubular Zn levels varied with age and demonstrated an accumulation pattern more similar to those for cortical tubular and cortical Cd and Cu. The levels of cortical tubular Zn in Group 4 were significantly higher than those for all other groups at 12 months and overall for the study as a whole. In addition, Group 4 was significantly higher than the Controls at 15 months and than Groups 2 and 3 at 21 months.

Thus, the data indicate that the overall effect of Cd exposure was to elevate the Zn and Cu concentrations above Control levels, in both the cortical tubules and the intact cortex. The effect on copper was much more dramatic, showing a 3- to 4-fold increase over Control levels for the highest cadmium dose as compared to a less than 2-fold increase of Zn over Control levels. Calculation of correlation coefficients between renal Cd and Cu concentrations and Cd and Zn concentrations indicated that there was a much stronger relationship between the levels of Cd and Cu than between the levels of Cd and Zn. These correlation coefficients for both cortical tubular and cortical metals are presented in Table 4-1. With the exception of cortical Zn, the concentrations of all other cortical and cortical tubular metals peaked at 15 months and decreased thereafter. For this reason, the correlation coefficients were calculated for both 0-15 and 0-24 months.

As indicated in Table 4-1, there was a significant, strong correlation between cortical Cd and Cu for Groups 3 and 4 and for the overall study as a whole. This was seen for both the 0-15 and 0-24 month periods. In contrast, there was no significant correlation between cortical Cd and Zn for any of the individual dosage groups, although there was a significant but weak correlation for the groups as a whole. The findings from these correlation coefficients support the graphical findings presented in Figures 4-10A, -B. Thus, there was a stronger, more linear relationship between increasing concentrations of renal Cd, and therefore increasing dose, and increasing levels of renal Cu. The association between levels of renal Cd and Zn was more subtle. Table 4-1 further shows that with the exception of Group 4 at 0-24 months, there was no significant correlation between the levels of cortical Cu and Zn at $p \leq 0.05$. Each of the Cd exposure groups had an inverse but insignificant relationship between cortical Cu and Zn; however, the Controls and the overall data showed a weak, direct relationship between these two metals.

The correlation coefficients, as well as the graphs of the group mean metal concentrations, show that the relationship between the three metals is different for the cortex as compared to the cortical tubules. As indicated in Table 4-1, there was a significant, strong correlation between tubular Cd and Cu for each of the Cd test groups and for all the groups as a whole. In contrast to the situation for the cortex, there was a significant, positive correlation between cortical tubular Cd and Zn for Groups 3 and 4 at 0-15 months and for Groups 2, 3 and 4 at 0-24 months. Additionally, there was a significant, positive correlation between cortical tubular Cd and Zn overall for both 0-15 and 0-24 months. For the time periods of 0-15 and 0-24 months, the overall Cd:Zn tubular correlation coefficients (0.47 and 0.48, respectively) were larger than the overall correlation coefficients for the cortices (0.36 and 0.39, respectively). Comparison of the correlation coefficients for cortical tubular Cd and Cu versus tubular Cd and Zn shows that generally the

Table 4-1: Correlation Coefficients Describing the Relationship Between Cadmium, Copper and Zinc in the Intact Renal Cortex and Cortical Tubules

	0 - 15 Months					0 - 24 Months				
	Control	Group 2	Group 3	Group 4	Overall	Control	Group 2	Group 3	Group 4	Overall
Cortex										
Cd:Cu	-0.24	0.33	0.54*	0.70*	0.77*	-0.11	0.13	0.42*	0.40*	0.63*
Cd:Zn	0.04	0.33	0.01	0.09	0.36*	0.07	0.27[b]	0.009	0.13	0.39*
Zn:Cu	0.28	-0.11	-0.07	-0.32[b]	0.13[a]	0.13[a]	0.26[c]	-0.03	-0.32*	0.20
Tubules										
Cd:Cu	0.10	0.44*	0.58*	0.58*	0.68*	-0.01	0.44*	0.59*	0.52*	0.59*
Cd:Zn	0.22	0.30	0.57*	0.36*	0.47*	0.22	0.38*	0.62*	0.52*	0.48*
Zn:Cu	-0.10	0.51*	0.27	0.61*	0.60*	0.33*	0.60*	0.46*	0.70*	0.67*

*Significant correlation coefficient at $p \leq 0.05$.

[a] Significant correlation coefficient at $p = 0.067$.

[b] Significant correlation coefficient at $p = 0.085$.

[c] Significant correlation coefficient at $p = 0.060$.

At each sacrifice, data were collected on 6 to 8 rats per test group. Therefore, the correlation coefficients calculated for 0-15 months were based on 38 to 48 rats per group while those calculated for 0-24 months were based on 56 to 72 rats per group. These figures include the 8 rats sacrificed at the zero time point.

"Overall" correlations were calculated by combining all the renal data, irrespective of exposure group, for either 0-15 or 0-24 months. For 0-15 months, the correlation coefficients were based on the data for 128 to 168 rats. For 0-24 months, the coefficients were based on the data for 200 to 264 animals.

relationship between Cd and Cu was still stronger than that between Cd and Zn, particularly for the 0-15 month interval. Finally, in contrast to the situation for the cortex, there appeared to be a significant, strong correlation between Zn and Cu in the cortical tubules.

The relative rates of cortical and cortical tubular accumulation of each of the three metals were calculated for each group for up to 15 months. The linear regressions of Zn, Cd and Cu were determined on a molar basis, against time of exposure and the slopes (expressed as moles of metal/gram dried tissue/unit of time) were compared. Most notably, in the 50 μg/ml exposure group, tubular Zn and Cu accumulated at roughly the same rates, on a molar basis; however, this rate was only about one-half the rate of cortical tubular Cd accumulation. Thus, the relative rates of cortical tubular accumulation, in Group 4, of Cd, Cu and Zn were 2:1:1, respectively, on a molar basis.

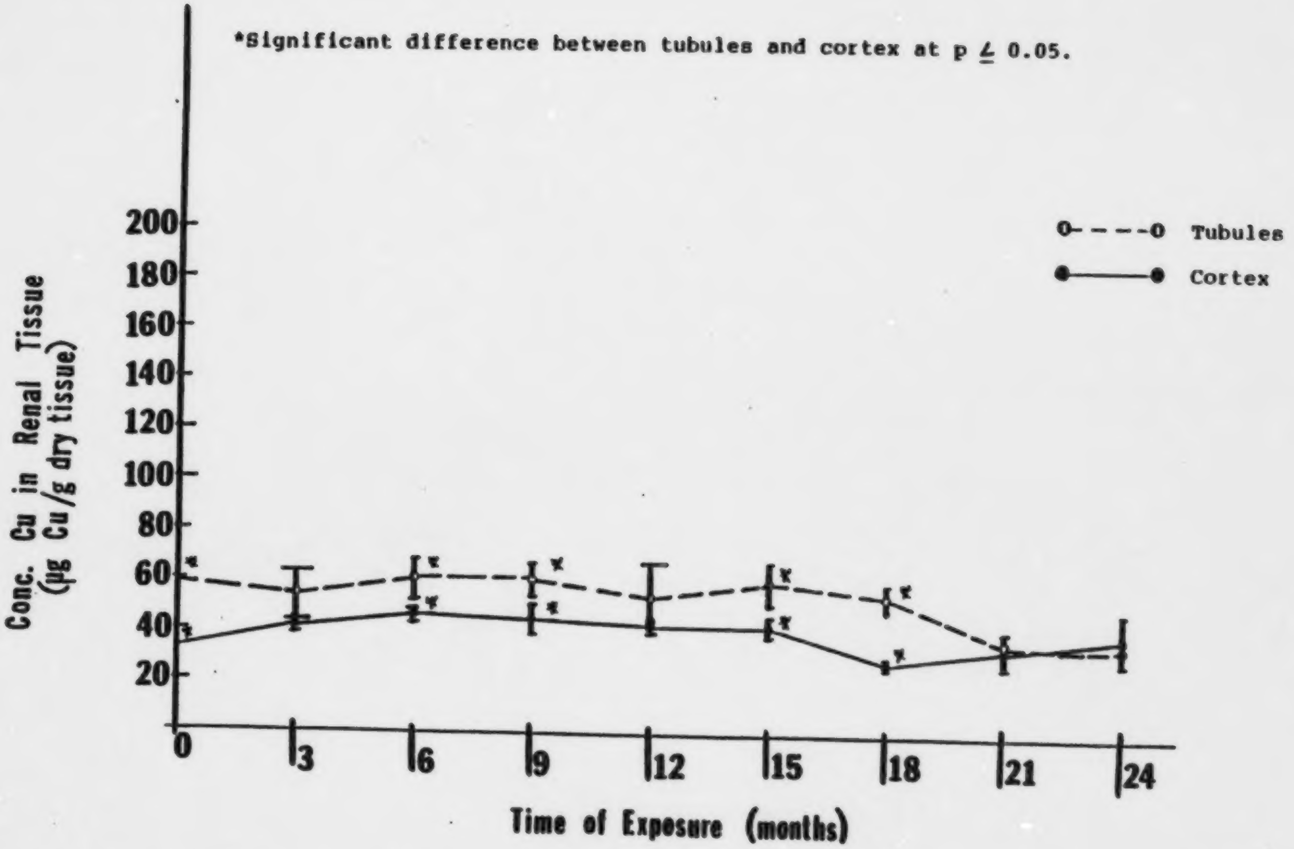
The concentrations of copper and zinc in the intact cortex as compared to the cortical tubules are illustrated in Figures 4-12, A-D and 4-13, A-D. All data are presented as μg of metal per gram of dry tissue and each point represents the mean ± S.E. for the tissues of 6 to 8 rats. At each sacrifice point, individual paired t-tests were performed on the data from each rat within a group to determine if there was a significant difference between the metal concentrations in the whole cortex versus the cortical tubules. The results of this statistical analysis are indicated in Figures 4-12 and 4-13. As demonstrated in these figures, the levels of both zinc and copper were significantly higher in the cortical tubules than in the cortices for both normal and Cd-dosed rats, at least through the 18th month. Thus, the cortical tubules preferentially accumulated not only Cd, but also Cu and Zn, relative to the whole cortex.

In a situation similar to that seen for Cd accumulation (Fig. 4-9), the levels of Cu and Zn in the cortical tubules decreased rapidly after 15-18 months. The rates of decrease in Zn and Cu concentrations were greater in the cortical tubules than in the intact cortices, with the result that after 18 months, the levels of tubular metals were less than the levels of cortical metals. By 24 months, the levels of tubular copper and zinc were significantly less than the levels of cortical copper and zinc for Groups 2, 3 and 4 (Fig. 4-12, B-D and Fig. 4-13, B-D, respectively). In the Controls, by 24 months, the levels of cortical tubular copper were lower than the levels of cortical copper, but the difference was not significant, and the levels of tubular and cortical zinc were equivalent.

With the exception of Group 4 cortical copper levels, by 24 months, both cortical and cortical tubular copper and zinc from all experimental groups had decreased to within normal values (see Tables A-6 to A-9). In contrast, by 24 months, the levels of both tubular and cortical Cd in Group 4 were still significantly elevated above Control levels (see Tables A-4, -5).

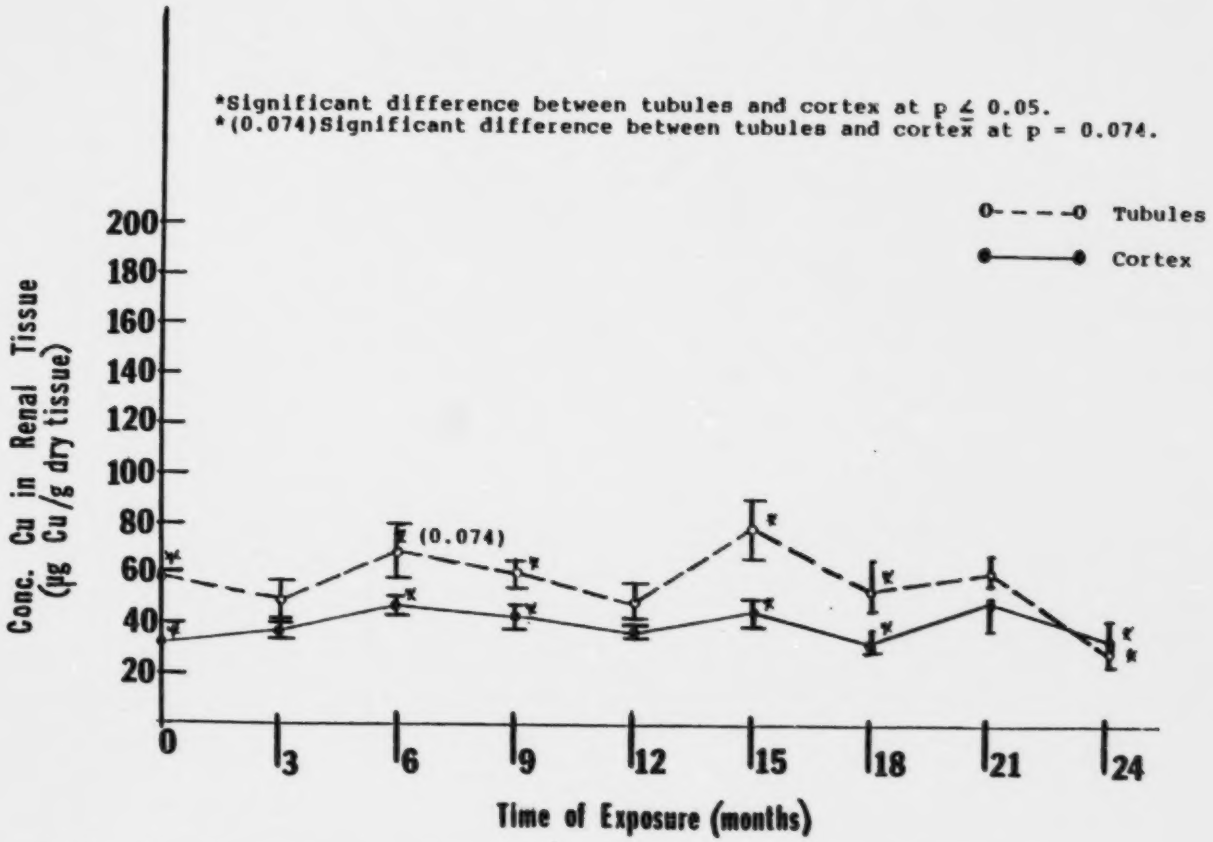
EFFECT OF AGE ON METAL ACCUMULATION IN THE KIDNEY

As shown in Figures 4-7 and 4-8, Cd concentrations in both the cortical tubules and intact cortices increased with time of exposure for all groups up to 15 months and then began to decrease. The same pattern was seen in the



*Significant difference between tubules and cortex at $p \leq 0.05$.

Figure 4-12A. Concentration of Copper in the Intact Renal Cortex and Cortical Tubules as a Function of Age in the Control Group



*Significant difference between tubules and cortex at p ≦ 0.05.
*(0.074)Significant difference between tubules and cortex at p = 0.074.

Figure 4-12B. Concentration of Copper in the Intact Renal Cortex and Cortical Tubules as a Function of Exposure to Cadmium in Group 2 (0.5 µg Cd/ml)

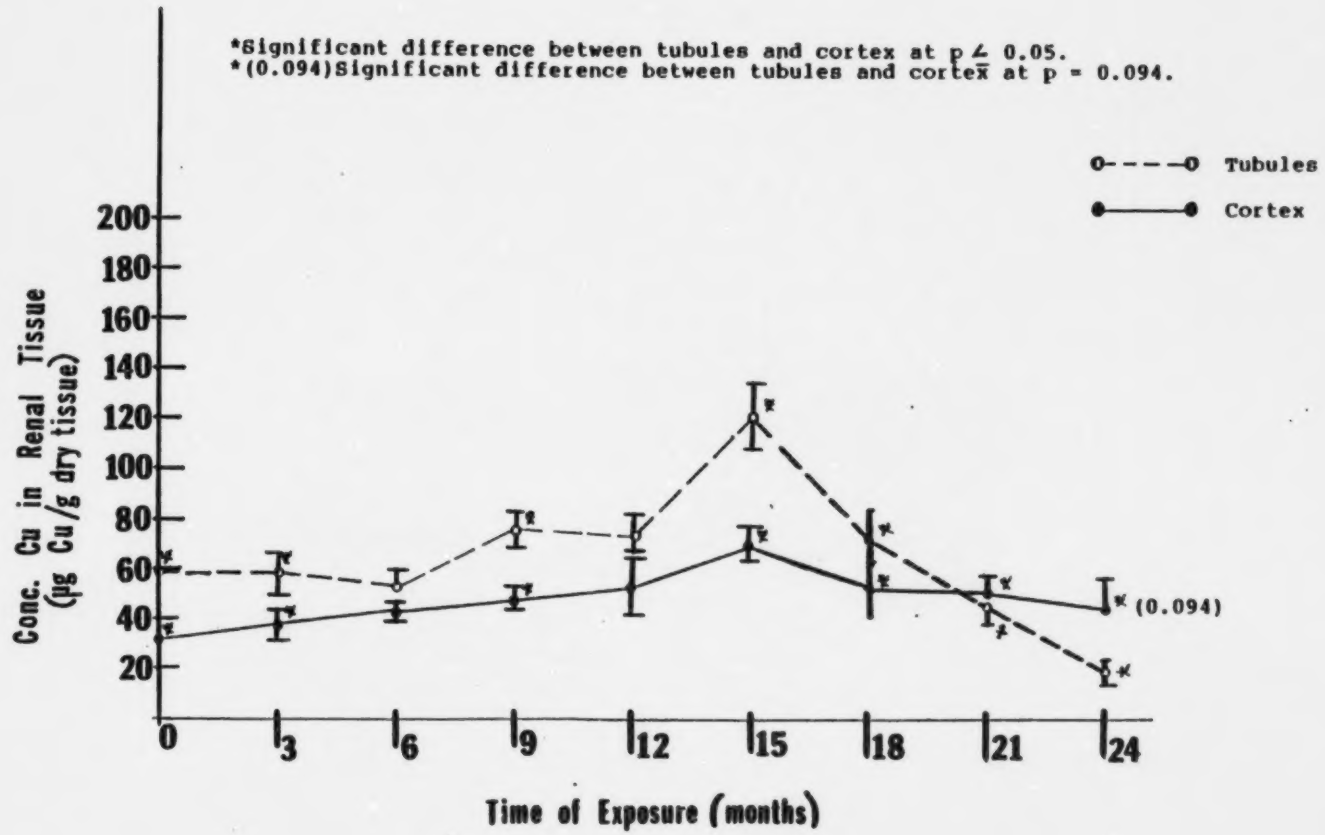
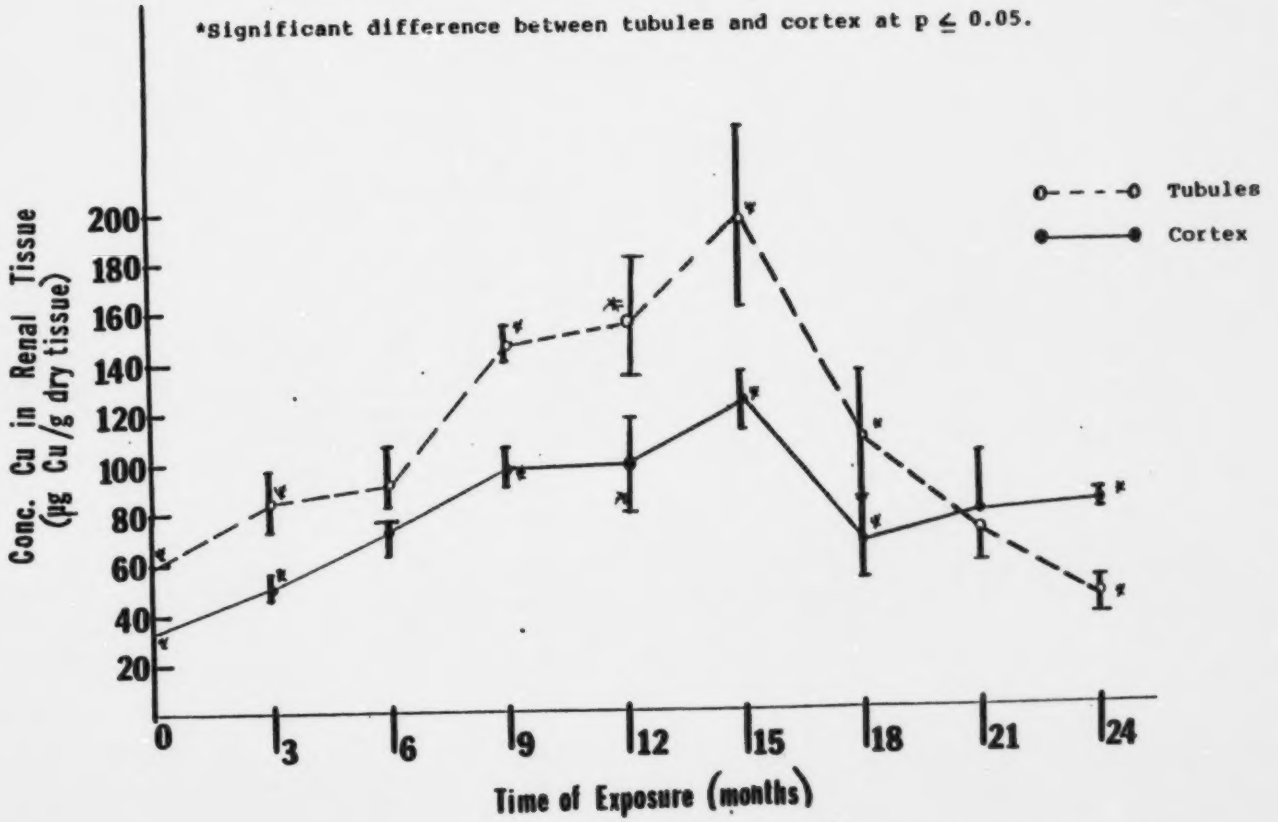


Figure 4-12C. Concentration of Copper in the Intact Renal Cortex and Cortical Tubules as a Function of Exposure to Cadmium in Group 3 (5.0 µg Cd/ml)



*Significant difference between tubules and cortex at $p \leq 0.05$.

Figure 4-12D. Concentration of Copper in the Intact Renal Cortex and Cortical Tubules as a Function of Exposure to Cadmium in Group 4 (50 µg Cd/ml)

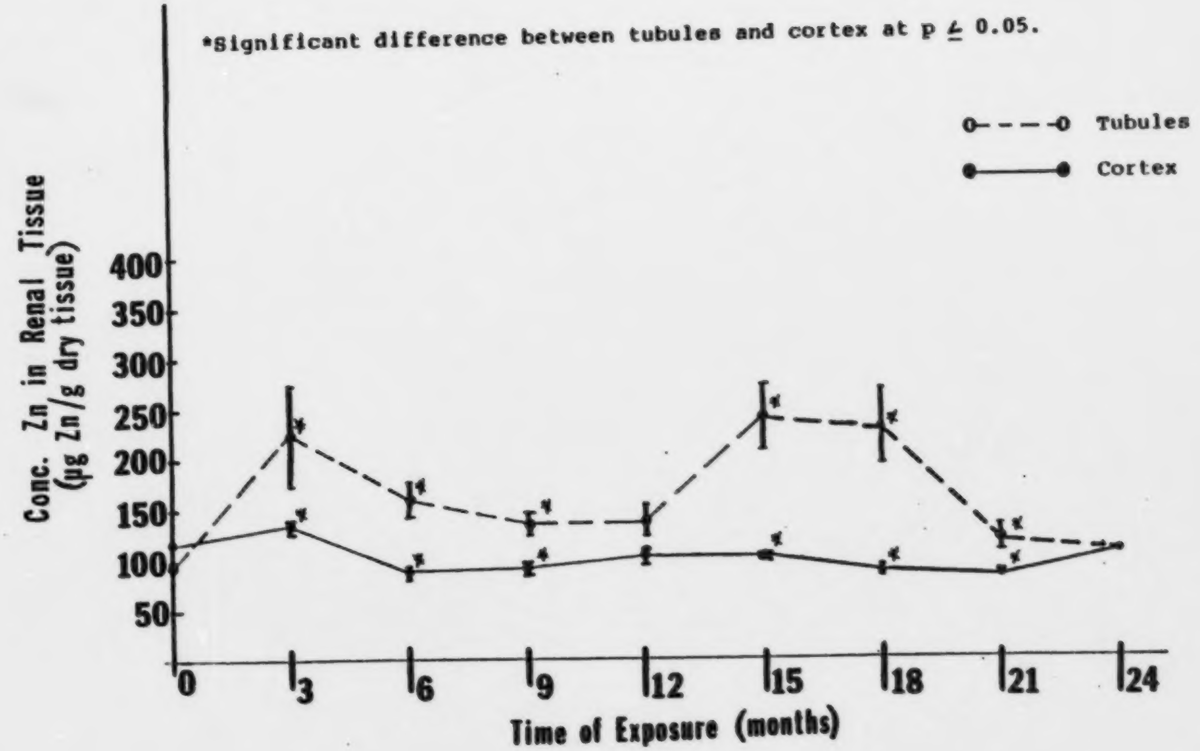


Figure 4-13A. Concentration of Zinc in the Intact Renal Cortex and Cortical Tubules as a function of Age in the Control Group

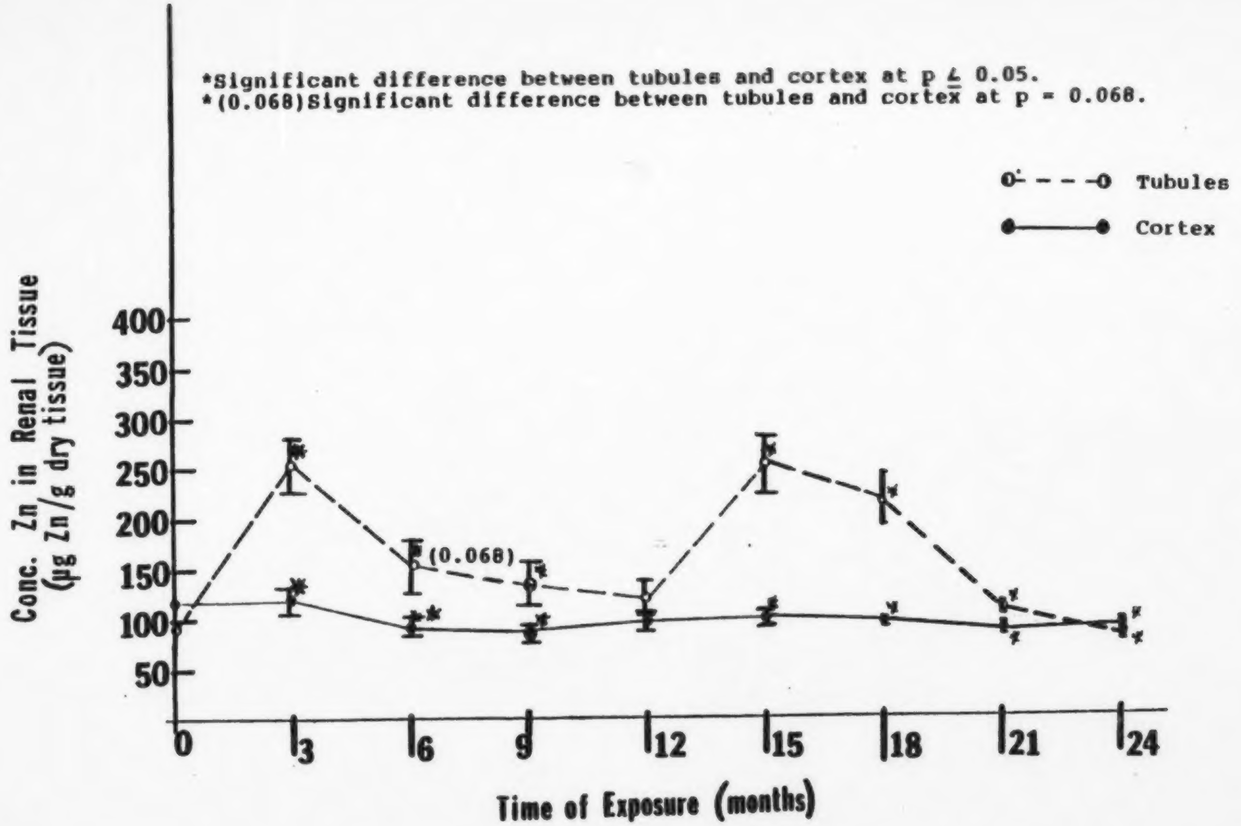
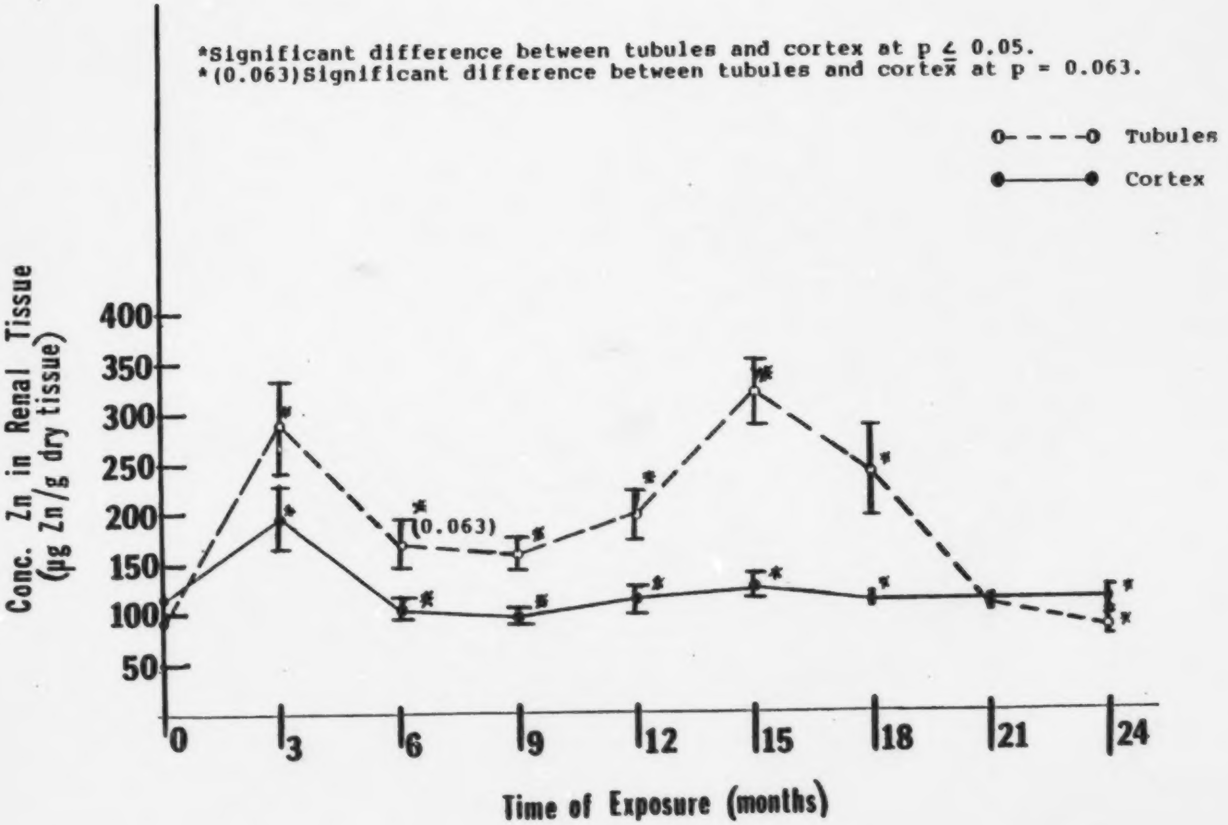


Figure 4-13B. Concentration of Zinc in the Intact Renal Cortex and Cortical Tubules as a Function of Exposure to Cadmium in Group 2 (0.5 µg Cd/ml)



*Significant difference between tubules and cortex at $p \leq 0.05$.
*(0.063)Significant difference between tubules and cortex at $p = 0.063$.

Figure 4-13C. Concentration of Zinc in the Intact Renal Cortex and Cortical Tubules as a Function of Exposure to Cadmium in Group 3 (5.0 µg Cd/ml)

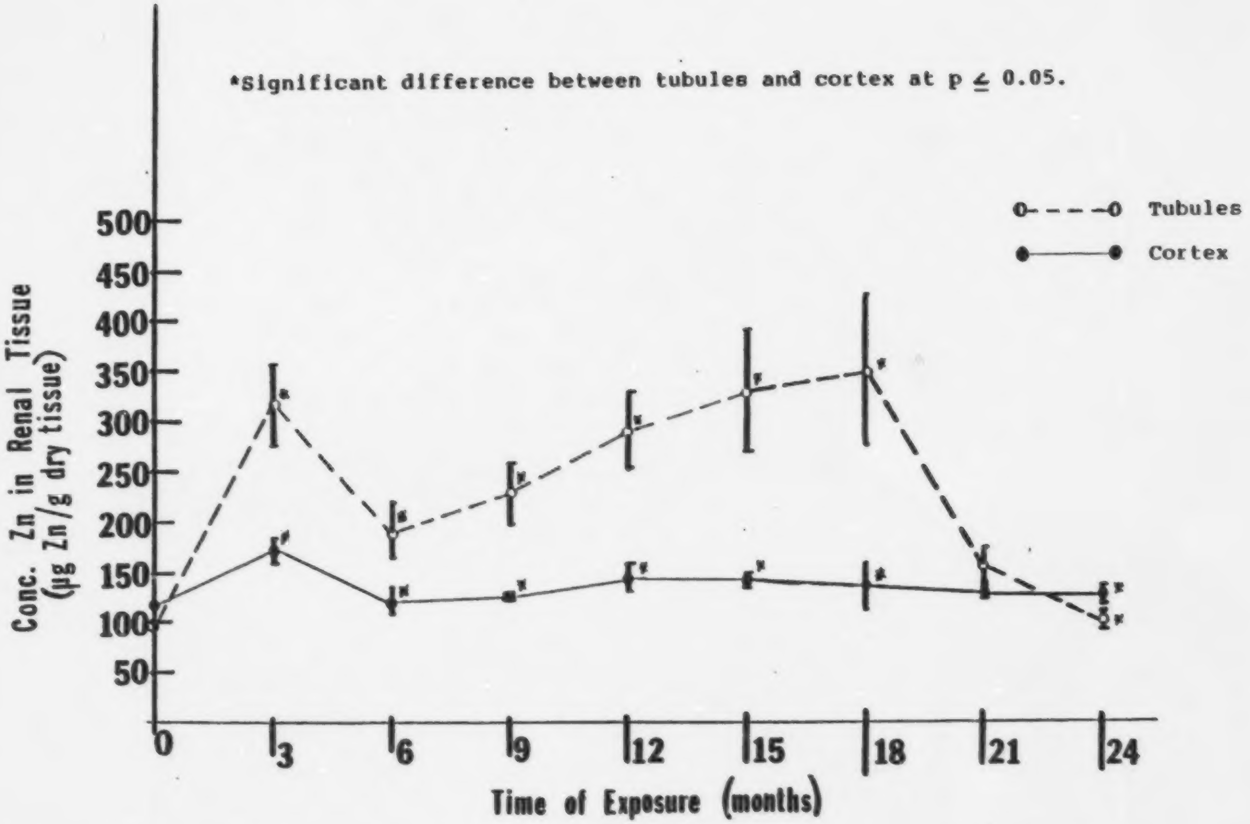


Figure 4-13D. Concentration of Zinc in the Intact Renal Cortex and Cortical Tubules as a Function of Exposure to Cadmium in Group 4 (50 μg Cd/ml)

Controls (Tables A-4 and -5). Even though many Control animals had Cd levels below the detection limits of the atomic absorption spectrophotometer, the group mean values still showed a trend towards peaking at 15 months. This time point of 15 months appeared to be a critical time for Cd kinetics independent of renal Cd burden, suggesting that the effect was related more to aging than to Cd toxicity.

As illustrated in Figures 4-7 and -8, 4-10A and -B, and 4-11A and -B, the accumulation patterns for each metal were quite different for the cortical tubules as compared to the whole cortex. A possible age-related effect was demonstrated to a far greater extent in the cortical tubules than in the whole cortex. In reference to the tubules, there was an initial rapid accumulation, up to the third month, of Cd and Zn in all groups and Cu in Group 4. This early, rapid accumulation phase occurred during the period when the rats were ingesting the largest amounts of Cd, on a μg Cd/day/kg rat weight basis, as shown in Figure 4-4. It also coincided with the period of most rapid growth in the kidney. As noted in Figure 4-22, the weight of the kidneys increased most rapidly between 0 and 3 months and then continued increasing gradually from 3 to 24 months. From 3 to 12 months, concentrations of Cu and Cd in the Control and lower dose groups remained stable, while those in Group 4 continued to increase but at a rate less than that observed during the first 3 months. Zn levels in all groups decreased between 3 and 6 months but then increased again through 12 months. As with Cu, the rate of increase in Zn between 6 and 12 months was less than the rate of Zn accumulation up to 3 months.

Group 4 tubules tended to sequester each of the three metals at constant rates from 6 to 15 months, or to 18 months in the case of Zn. In contrast, the Control and both low dose groups had a significant increase in the tubular concentration of all metals, with the exception of Control Cu, that started at 12 months and peaked at 15 months. With the exception of Control Cu, the tubular concentrations of all three metals in all study groups started to decrease rapidly by 15 to 18 months. Thus, 15 months appeared to be a critical time point not only for Cd, but also for Cu and Zn kinetics.

ACCUMULATION OF HEPATIC CADMIUM, COPPER AND ZINC

Figures 4-14A-C illustrate the patterns of accumulation of hepatic cadmium, zinc, and copper, respectively. All data are expressed as μg metal/gram dry weight of liver. Each point represents the group mean value for 6 to 8 rats.

Analysis of variance showed there was a significant difference in hepatic cadmium levels between the early and late arriving rats at 0, 9, 12 and 15 months; however, there was no significant difference for the overall study. For hepatic copper levels, there was a significant difference between early and late rate only at 6 and 12 months, and no significant difference overall. Zinc concentrations differed significantly between the two sets of rats for 0, 3 and 12 months and for the overall study.

For the graphical presentation of the data (Fig. 4-14), the early and late arriving rats have been treated as one homogenous group. Thus, each data point represents the mean value of both the early and late rats within a single exposure group. Examination of the raw metal concentration data indicated

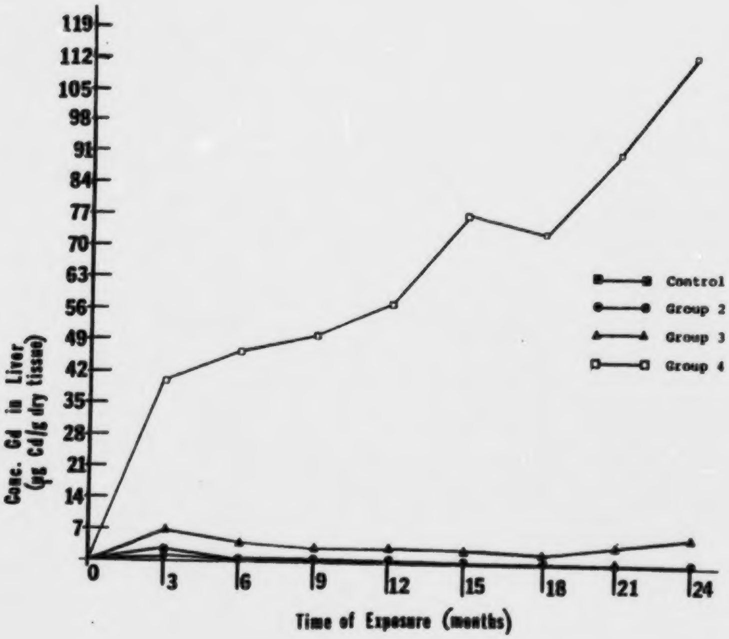


Figure 4-14A. Concentration of Hepatic Cadmium (μg Cd/g dry tissue) as a Function of Exposure to Cadmium

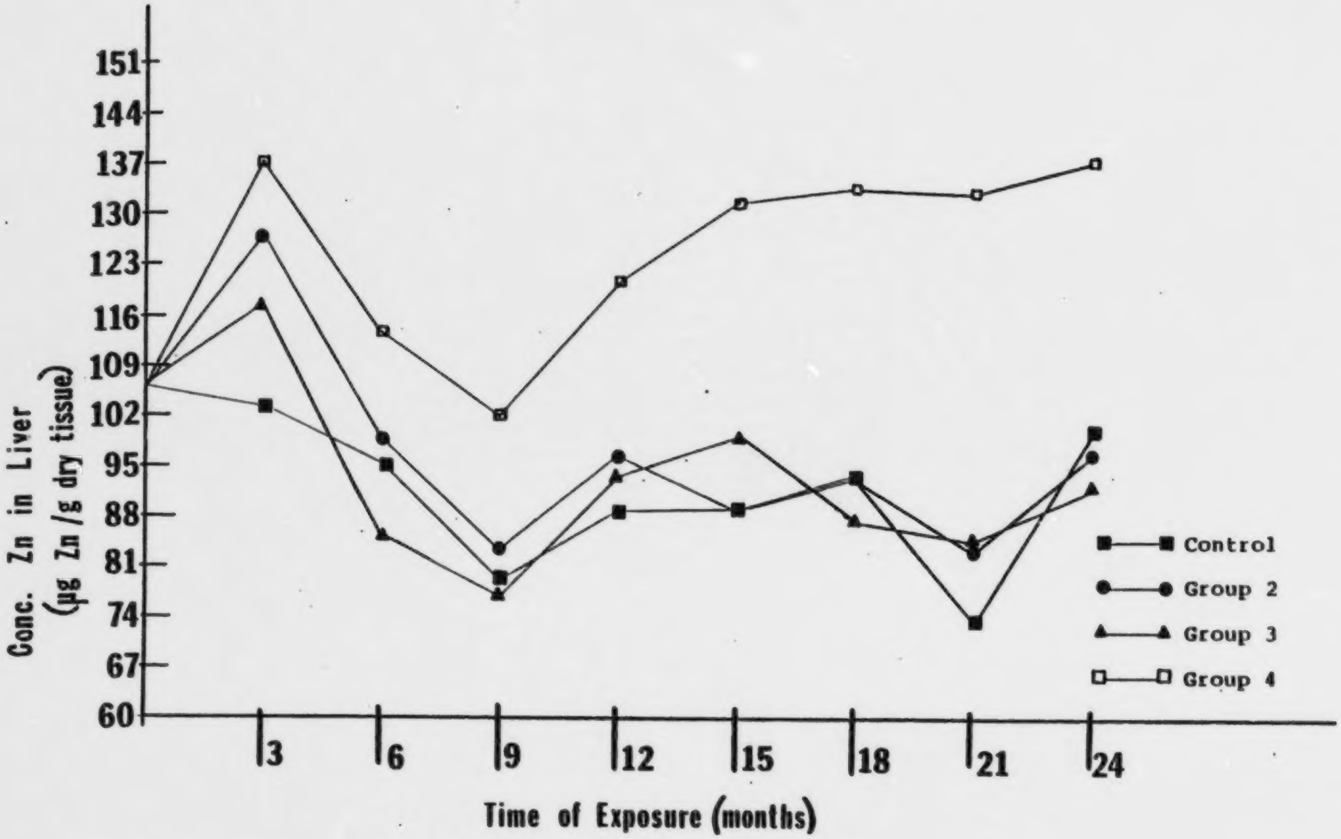


Figure 4-14B. Concentration of Hepatic Zinc (μg Zn/g dry tissue) as a Function of Age and Exposure to Cadmium

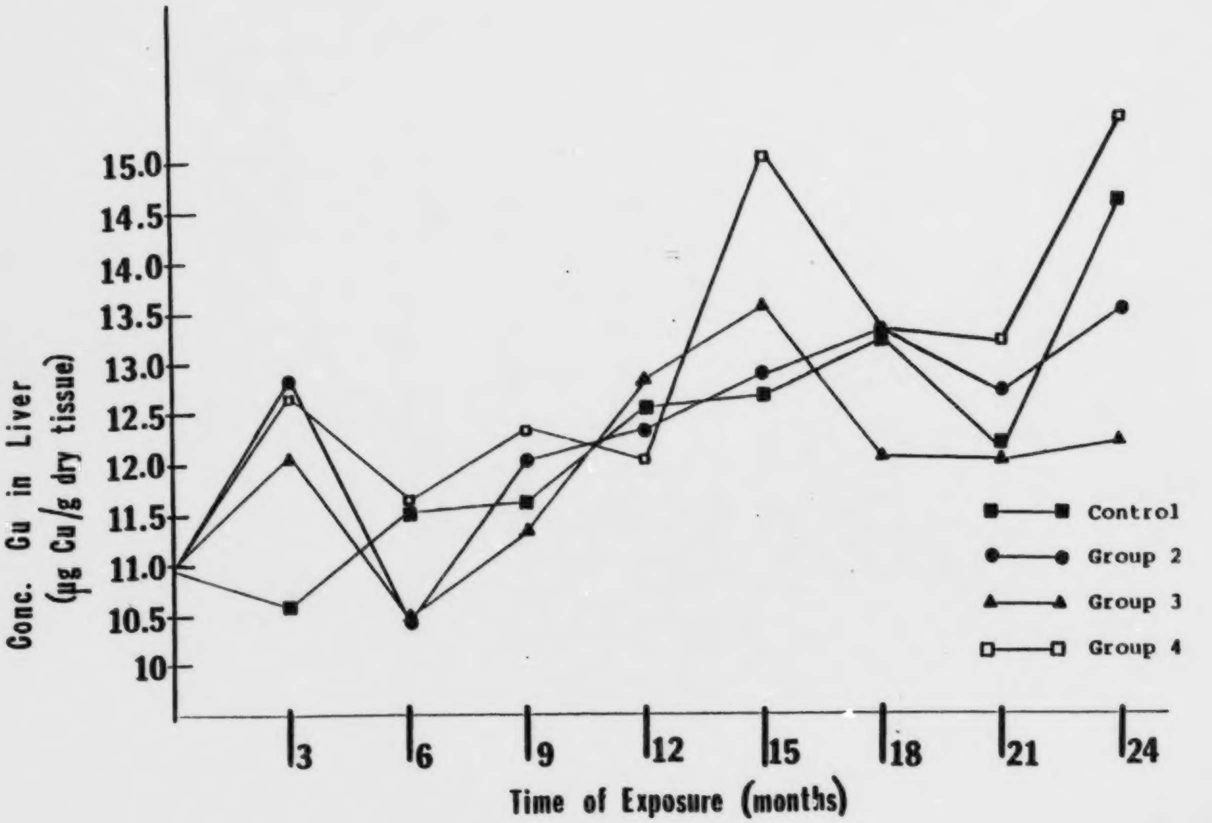


Figure 4-14C. Concentration of Hepatic Copper (µg Cu/g dry tissue) as a function of Age and Exposure to Cadmium

that, although the two sets of rats differed significantly at certain time points, these differences were not large enough to actually affect the shapes of the curves.

Tables A-10-12 present the results of the ANOVA for determining the significance of differences between group mean values for hepatic cadmium, zinc, and copper, respectively.

The data indicate that there was a definite dose-response relationship between the levels of ingested Cd and the levels of hepatic Cd and Zn. At each sacrifice point, and for the overall study, Group 4 hepatic Cd concentrations were significantly higher than the concentrations in all other groups. Figure 4-14A shows that there also was a consistent trend towards elevated hepatic Cd in Group 3, as compared to the Controls and Group 2; however, this difference was not statistically significant. The levels of Cd in Group 2 also consistently exceeded those in the Control group, but again the difference was not significant (Table A-10).

Similar to the accumulation pattern of renal Cd, there was an initial rapid rate of accumulation of hepatic Cd in all groups up to 3 months. As noted previously, the first 3 months corresponded to the time when the rats were ingesting the highest concentrations of Cd, on a µg Cd/day/kg rat weight basis (Fig. 4-4). Moreover, the liver almost doubled in weight from 0 to 3 months and then remained stable in size through 24 months. For Group 4, the rate of accumulation of hepatic Cd remained constant from 3 to 15 months, but then increased again from 18 to 24 months. As indicated in Figure 4-14A, the concentration of Cd in this high dose group never stabilized. In contrast, Groups 2 and 3 levels remained constant (i.e., the fluctuations were significant at $p \leq 0.05$) from 6 months to the end of the study.

Figure 4-14B shows that Cd exposure had little consistent effect on elevating Group 2 or 3 hepatic Zn levels above the Control values. The greatest effect was seen in Group 4, where the levels of Zn were significantly higher ($p \leq 0.05$)than all other groups at 9, 15, 18, 21, 24 months. Although the Group 4 levels were significantly elevated throughout the entire study, the shapes of the hepatic Zn curves were similar for all four groups. With the exception of the Controls, the three dosed groups had an initial, rapid accumulation of Zn in the first 3 months. Between 3 and 9 months, the levels of Zn in all groups decreased. Between 9 and 15 months, there was another rise in all groups, followed by a general stabilization of levels through 24 months. Thus, the graphs for all four groups indicate similar changes of hepatic Zn levels with age, irrespective of the size of the Cd dose.

Finally, Figure 4-14C shows the accumulation patterns of hepatic copper. In contrast to hepatic Zn and Cd, there was no dose response relationship with hepatic Cu levels. As indicated in Table A-12, Group 4 was significantly higher than Group 3 at 24 months ($p \leq 0.05$) and overall ($p = 0.01$); otherwise there were no significant differences between any other groups. With the exception of Group 3 from 15-24 months, there appeared to be a gradual, yet significant, increase in hepatic copper levels in all groups with increasing age.

To further examine the relationship between the levels of hepatic Cd, Zn and Cu, correlation coefficients were determined for each pair of metals. Also, the correlation between renal Cd and hepatic Cd was calculated. All correlation coefficients were calculated using the tissue concentrations on a dry weight basis (i.e., µg metal/g dry tissue) and are presented in Table 4-2. Since renal Cd levels in all groups peaked at 15 months, the correlation of renal Cd with hepatic Cd was calculated for both 0-15 and 0-24 months. For each pair of variables, the correlation coefficient was determined by exposure group and then overall for all the data, irrespective of group.

Table 4-2: Correlation Coefficients for Hepatic Cd:Zn, Cd:Cu, Zn:Cu and Renal Cd:Hepatic Cd for 0-24 Months

	Control	Group 2	Group 3	Group 4	Overall
Hepatic Cd:Cu	0.04	0.13	0.11	0.47*	0.29*
Hepatic Cd:Zn	0.01	0.50	0.11	0.62*	0.60*
Hepatic Zn:Cu	0.29*	0.22[a]	0.17	0.39*	0.38
Renal Cd:Hepatic Cd	-0.0004	-0.24	-0.24[b]	0.55*	0.92*
Renal Cd:Hepatic Cd[c]	-0.03	-0.43	-0.20	0.68*	0.95*

*Significant correlation coefficients at $p \leq 0.05$.

[a] Significant correlation coefficients at $p = 0.088$.

[b] Significant correlation coefficients at $p = 0.079$.

[c] Correlation coefficients calculated for 0-15 months.

See Table 4-1 for calculation of the number of rats used for determining correlation coefficients.

As indicated in Table 4-2, there was a strong, positive correlation between the levels of hepatic Cd and hepatic Zn. This relationship was significant for Groups 2 and 4 and overall. In contrast, the correlation between hepatic Cd and hepatic Cu was significant for Group 4 and overall, but was much weaker than the correlation between Cd and Zn. The magnitude and significance of the correlation coefficients support the findings presented in Figure 4-14A-C, in that increases in hepatic Cd are more closely related to increases in hepatic Zn as opposed to increases in hepatic Cu. The correlation coefficients also show that there was a weak, though none-the-less significant, trend toward increased hepatic Zn paralleling increased hepatic Cu concentrations.

Since the kidney and liver together from the largest reservoir for tissue cadmium in the body, it was of interest to examine the relationship between the levels of renal and hepatic cadmium. As noted in Table 4-2, with the exception of Group 2, the correlation coefficients were similar when calculated for 0-15 and 0-24 months. The data indicate that, in general, for the Control and low dose groups, there was a trend towards a weak inverse relationship between renal and hepatic Cd accumulation. However, with the exception of Group 2 for 0-15 months, this trend was not significant at $p \leq 0.05$. In contrast, the high dose group had a significant, positive and fairly strong correlation between renal and hepatic Cd accumulation. Moreover, when all the data were examined, irrespective of group, there was an almost perfect correlation between renal and hepatic Cd levels, indicating that renal Cd concentrations increased linearly with hepatic Cd concentrations.

KIDNEY AND LIVER CADMIUM ORGAN BURDENS

The total accumulated amounts of Cd, as µg Cd/organ, were calculated for both the kidneys and liver for Groups 2 and 3 (Fig. 4-15A) and Group 4 (Fig. 4-15B). At each sacrifice, the mean hepatic Cd burden was calculated for each group as follows: µg Cd in liver = (group mean liver wet weight, g)•(group mean hepatic Cd concentration, µg Cd/g wet weight).

The concentration of renal Cd was measured only for the kidney cortex and did not include measurements for the medulla or for the whole kidney. Therefore, calculations of the total kidney Cd burden were based on the assumption that the concentration of Cd in the cortex was 1.5 times greater than the concentration in the whole kidney (Friberg, *et al.*, 1974; Kawai *et al.*, 1976). At each sacrifice, the mean renal Cd organ burden was calculated for each group as follows:

µg Cd in 2 kidneys =
[2•(group mean whole kidney wet weight, g)•(group mean cortex Cd concentration, µg Cd/g wet weight)] ÷ 1.5

Figure 4-15 shows that the relationship between the accumulated kidney and liver Cd organ burdens differed among the exposure groups. From the lowest to the highest dosage group, there was a trend toward the liver Cd burden exceeding the renal Cd burden. Thus, in Group 2, total hepatic Cd exceeded total renal Cd from 0-6 months. In Group 3, hepatic Cd exceeded renal Cd from 0 to almost 12 months and again from about 20 months to the end of the study. In Group 4, the hepatic Cd burden was significantly greater than the renal burden for the entire study period.

Since the liver and kidneys together form the body's major cadmium reservoir, these data suggest that the partitioning of the Cd body burden into renal and hepatic fractions was dose dependent. Thus, with increasing dose, a larger proportion of the total Cd body burden was sequestered in the liver as opposed to the kidneys. Further, age appeared to be a factor which had a slightly different effect, depending on dose. First, in all groups, except Group 2 kidneys, the rate of accumulation of Cd in both the liver and the kidneys was highest from 0-3 months. This coincided with the time of most rapid increase in liver and kidney weight. Thus, for all groups, the mean liver wet weight increased significantly up to 3 months (i.e., doubling in weight) and then remained stable through 24 months (Table A-32). The kidney mean wet weight in all groups also increased very rapidly up to 3 months but then continued increasing gradually through 24 months (Figure 4-22).

In Group 2, from 3 to 6 months, a noticeable decrease in the hepatic Cd burden coincided with a gradual increase in the renal burden. With the exception of the 12 to 18 month period, both renal and hepatic Cd levels

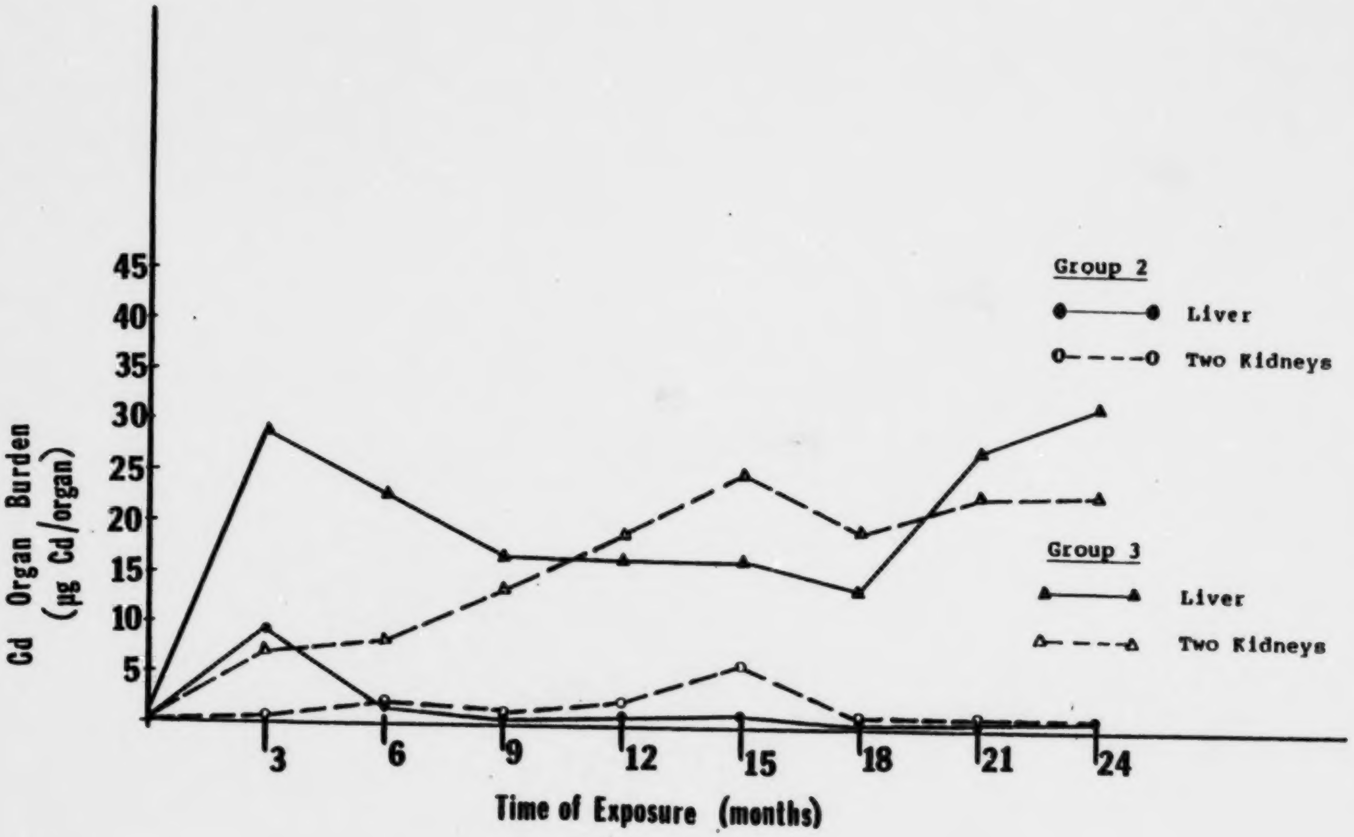


Figure 4-15A. Kidney and Liver Cadmium Organ Burdens (µg Cd/organ) for Group 2 (0.5 µg Cd/ml) and Group 3 (5.0 µg Cd/ml) as a Function of Exposure to Cadmium

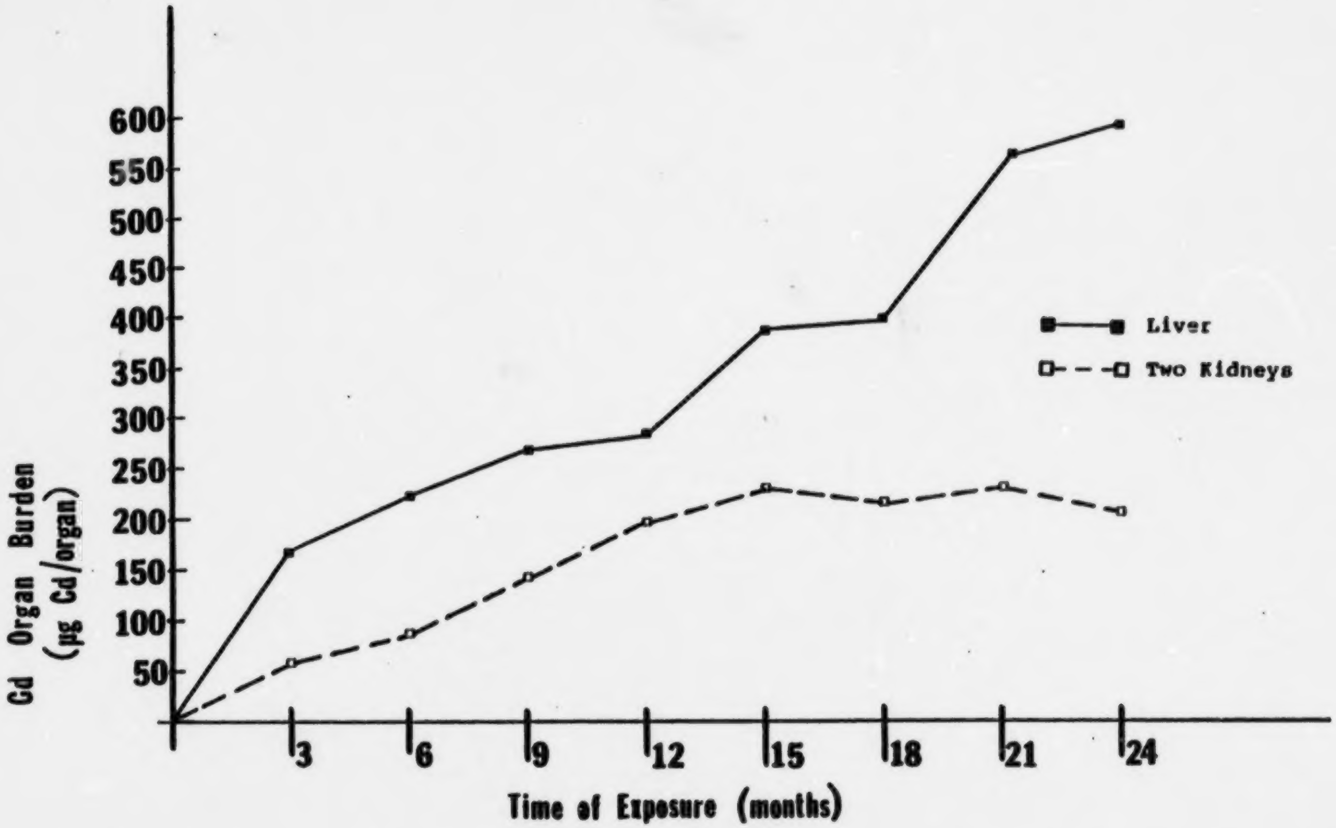


Figure 4-15B. Kidney and Liver Cadmium Organ Burdens (µg Cd/organ) for Group 4 (50 µg Cd/ml) as a Function of Exposure to Cadmium

remained stable. In contrast, in Group 3, the decline of hepatic Cd, from 3 to 18 months, coincided with a significant rise in renal levels from 0-15 months. After 18 months, Cd storage in the liver increased significantly again, while that in the kidney rose slightly. The situation in Group 4 was completely different from that in the lower exposure groups. As indicated in Figure 4-15B, after the initial 3 month increase, the amount of Cd in the liver increased in a linear fashion through 24 months. From about 3 to 15 months, the renal Cd burden increased at a rate almost paralleling that for the liver. After 15 months, the storage of renal Cd in Group 4 stabilized in a manner similar to that for Group 3.

Finally, as indicated previously in Figures 4-3 and 4-4, water consumption and therefore exposure dose, as µg Cd/day/kg rat weight, decreased rapidly from 0 through 6 months and then remained stable through the end of the study. It was of interest that in Group 2, both the liver and renal Cd burdens, with the exception of the 15 month kidneys, also remained stable from 6 months through the end of the study. In contrast, the Group 4 liver burden never stabilized, while that in the kidney plateaued after 15 months. Also, as noted previously, from 6 to 24 months, the actual Cd dose to the rats increased by a factor of 8 between Groups 2 and 3 and by a factor of 7.8 between Groups 3 and 4. For this same time period, the renal Cd burden in Group 3 exceeded that in Group 2 by an average factor of 13.34, with a range of values of 4.00 to 24.34. Further, the renal burden in Group 4 exceeded that in Group 3 by an average factor of 9.95, with a range of 8.75 to 10.92.

ACCUMULATION OF METALS IN THE INTESTINE, LUNG, MUSCLE, HEART AND SPLEEN

Tables A-13 through A-27 in the Appendix summarize the data concerning the accumulation patterns of Cd, Zn and Cu in the intestine, lung, muscle, heart and spleen. All values are expressed as the concentration of metal on a dry weight basis and each represents the mean of 6 to 8 rats within a dosage group. In addition, Figures 4-16A-C display the accumulation patterns in the intestine of Cd, Zn and Cu, respectively.

As seen in Table A-3, it was found that the early and late arriving rats differed significantly in their levels of intestinal Cd at 0, 9, 12, 18 months and overall, and they differed significantly in their levels of intestinal Zn at 0, 3, 15, 21 and 24 months but not overall. There were no significant differences in the levels of intestinal Cu except for the 0 month point. Graphical presentation of the intestine metals data (Fig. 4-16) has been handled in a manner similar to that for the renal metals. The early and late arriving rats have been treated as one homogeneous group, so that each data point represents the mean tissue metals concentration of 6 to 8 rats. Also, the results of the analysis of variance for determining significant differences between groups were not affected by the disparities between the early and late arriving rats.

As indicated in Table A-13 and Figure 4-16A, there was a clear dose-response relationship between levels of Cd exposure and the concentration of Cd in the intestine. Analysis of variance showed that from 6 through 24 months, and overall, Group 4 had significantly higher concentrations of intestinal Cd than all other groups. Additionally, at 3 months, Group 4 levels

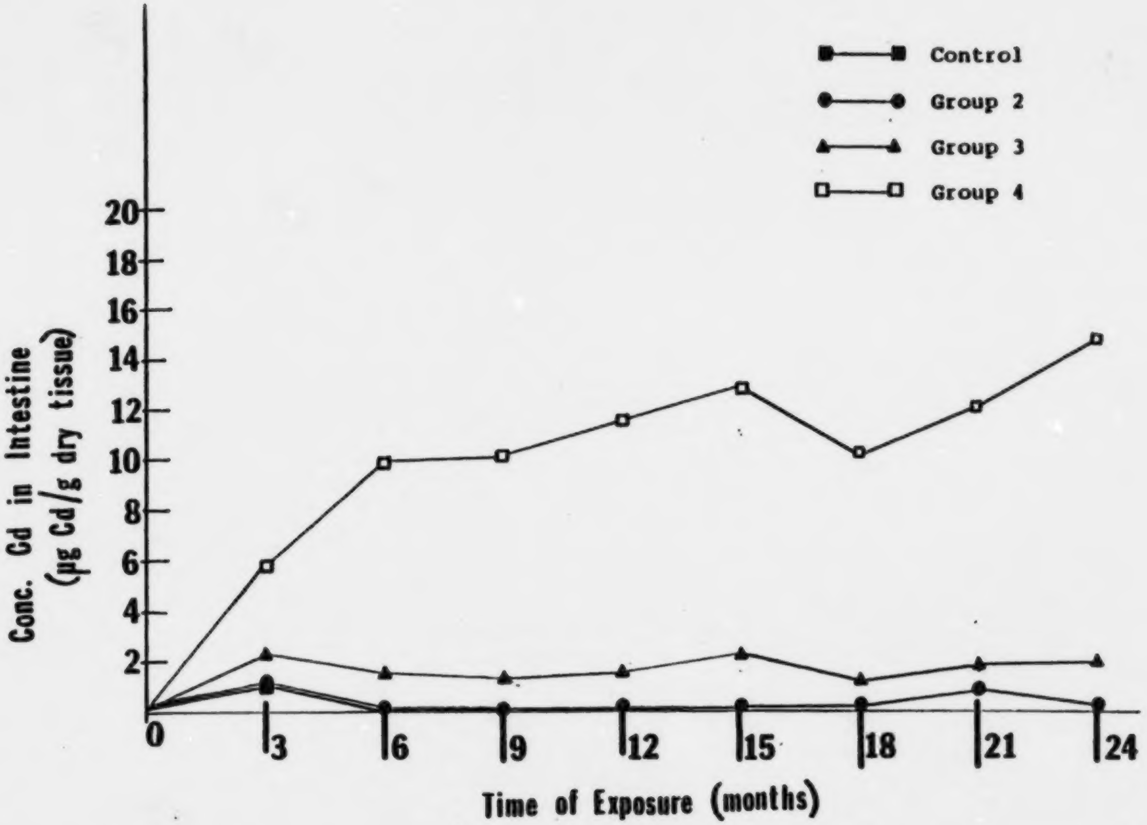


Figure 4-16A. Concentration of Cadmium in the Intestine (µg Cd/g dry tissue) as a Function of Exposure to Cadmium

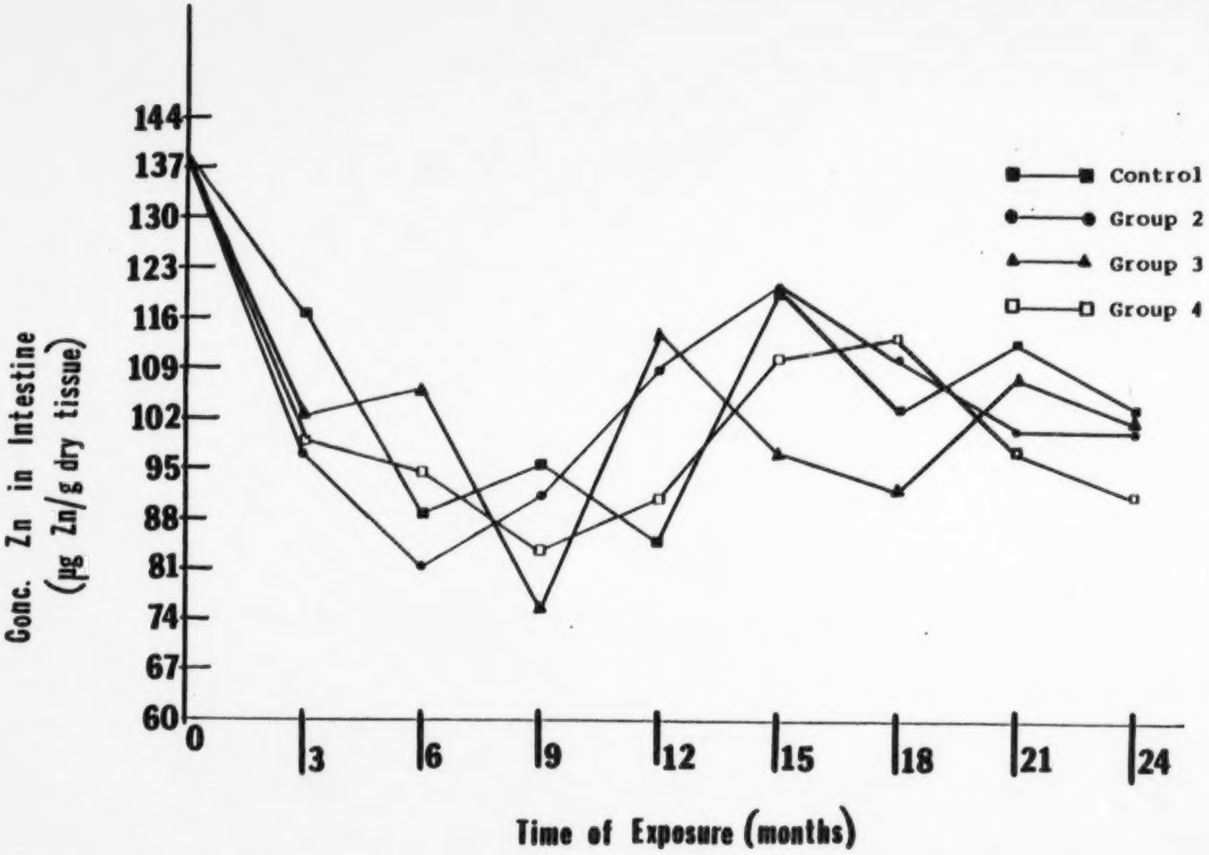


Figure 4-16B. Concentration of Zinc (µg Zn/g dry tissue) in the Intestine as a Function of Age and Exposure to Cadmium

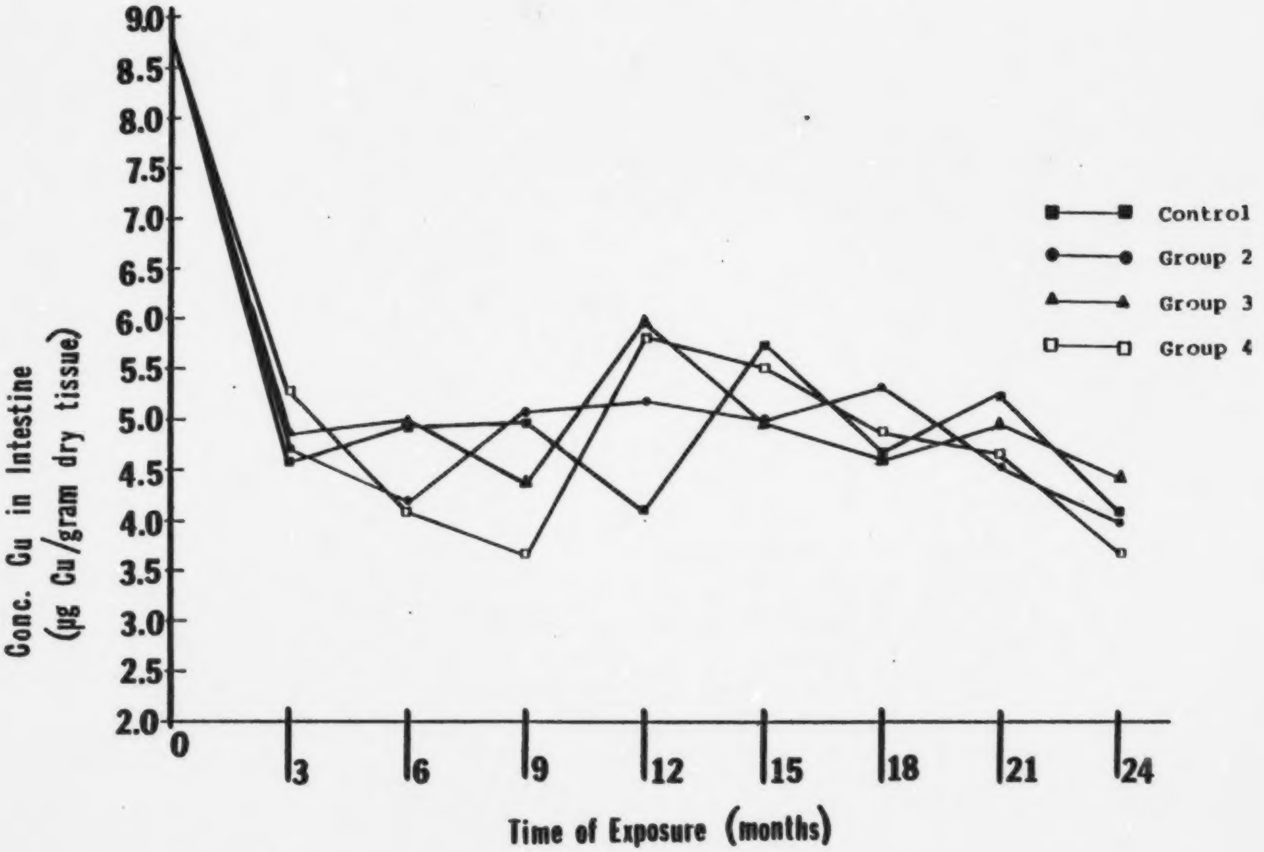


Figure 4-16C. Concentration of Copper (µg Cu/g dry tissue) in the Intestine as a Function of Age and Exposure to Cadmium

were significantly higher than those of Controls or Group 2 ($p = 0.10$). Although significant differences were not found among the Controls and two lower dose groups, Figure 4-16A shows that there was a trend towards increased tissue Cd concentration with increased dose. Similar to the liver and kidneys, the intestine appeared to accumulate Cd most rapidly during the first 3 months, when the animals ingested the highest doses of Cd. Unlike the liver and kidney, these first 3 months did not coincide with a period of rapid organ growth (Table A-28). In Groups 2 and 3, after a loss of Cd between 3 and 6 months, the concentrations generally stabilized for the remainder of the study. In Group 4, the major increase in Cd concentrations occurred through 6 months. Thereafter, the levels fluctuated, but plateaued. It was of interest that at 21 months, all three groups showed an increase in Cd concentrations which coincided with an unexplained late rise in water consumption (Fig. 4-3) and, therefore, a rise in exposure dose (Fig. 4-4). Group 4 intestinal Cd continued to rise through 24 months, as did water consumption.

In contrast to the situation in the liver and kidney, Cd exposure had no effect on elevating the levels of Zn or Cu in the intestine (Tables A-14, A-15, and Figs. 4-16B and -C, respectively). There appeared to be an effect of age, irrespective of Cd dose, on the levels of intestinal Zn. In general, the levels decreased from a high baseline concentration to a low at around 9 months and then increased again through 15 months. After 15 months, the concentrations fluctuated but levelled off. The graphs for Cu accumulation (Fig. 4-16C) generally follow the shape of the graphs for Zn accumulation; however, there was a more noticeable trend toward decreasing concentrations after 12 months.

Correlation coefficients calculated for 0-24 months for each group and for all the data irrespective of group showed that with the exception of Group 3 ($r = 0.33$, $p \leq 0.05$), there was no relationship between the levels of Cd and Cu in the intestine. Further, there was no relationship between the levels of Cd and Zn in any group or overall. In contrast, there was a significant, positive correlation between Zn and Cu levels in the intestine at $p \leq 0.05$ for all groups. The correlation coefficients were as follows: Controls, 0.30; Group 2, 0.59; Group 3, 0.38; Group 4, 0.36; overall, 0.44.

Tables A-16 through A-18 in the Appendix summarize the data concerning the concentrations in the lung of Cd, Zn and Cu, respectively. The concentration of Cd and Cu did not differ significantly between the early and late arriving rats; however, the Zn levels differed significantly at 3 and 18 months as well as overall.

As indicated in Table A-16, there was a dose-response relationship between Cd exposure and concentrations of Cd in the lung. From 9 through 24 months and overall, Group 4 had significantly higher metal concentrations than all other groups ($p \leq 0.05$). Additionally, at 6 months, Group 4 was significantly higher than the Controls and Group 2. Overall, Group 3 also was significantly higher than the Controls ($p = 0.10$).

Similar to the condition found in the intestine, Cd accumulation in the lung had no significant effect on the distribution of either Zn or Cu in the lung (Tables A-17 and A-18). Correlation coefficients calculated for 0-24 months showed that there were no significant relationships between the levels of Cd and Zn in the lung. With the exception of Group 2 ($r - 0.26$, $p \leq 0.05$), there were no significant relationships between Cd and Cu in the lung for any of the other groups, or for the overall data as a whole. There was a weak association between Zn and Cu, but this was found only in Group 4 ($r = 0.30$, $p \leq 0.05$) and for the overall data as a whole ($r = 0.27$, $p \leq 0.05$).

Tables A-19 through A-21 in the Appendix summarize the data on accumulation of Cd, Zn, Cu in the muscle. Early and late arriving rats differed significantly in Cd concentrations only at 6 and 24 months. There was no significant difference between the two sets of rats for Cu concentrations; however, there was a significant difference at 0, 3 months and overall for Zn concentrations (Table A-3).

As shown in Table A-19, only trace amounts of Cd accumulated in the muscle and there was no significant difference between any of the groups at any time. Further, as seen in Tables A-20 and A-21, Cd accumulation in the muscle had no effect on the distribution of either Zn or Cu. Although analysis of variance showed no significant group differences for Cu in the muscle, the correlation coefficients showed that there was a weak, but significant relationship between Cd and Cu in the muscles of the two low dose groups. Over the period 0-24 months, Group 2 ($r = 0.30$, $p \leq 0.05$) and Group 3 ($r = 0.36$, $p \leq 0.05$) demonstrated this relationship; however, a similar relationship was not detected in Group 4 or in the overall data as a whole. There was no relationship between the levels of Cd and Zn in the muscle. With the exception of the Controls ($r = 0.33$, $p \leq 0.05$), there also was no relationship between the levels of Zn and Cu in the muscle.

Tables A-22 through A-24 in the Appendix summarize the group data on concentrations of Cd, Zn and Cu in the heart. Since the metals analysis was performed on the hearts of the late arriving rats only, it was not known if there was a significant difference between the early and late arriving animals. As indicated in Table A-22, the heart, even in Group 4, accumulated only very small amounts of Cd. Although the levels of Cd in the hearts of Groups 2 and 3 were insignificant, the accumulation of Cd in this organ has shown a dose-response relationship with the ingested metal. At 3, 6, 15, 24 months and overall, Group 4 had significantly higher concentrations of Cd than all other groups.

Similar to the condition in the intestine, lung and muscle, the accumulation of Cd in the heart had no effect on the accumulation of either Zn or Cu in this organ (Tables A-23 and A-24). As expected, there was no correlation between the concentrations of Cd and Cu in the heart. Group 4 showed a significant relationship between the levels of Cd and Zn ($r = 0.43$, $p \leq 0.05$); however, this relationship was not found in any other group or in the data as a whole. In contrast, there was a rather strong relationship between the concentrations of Zn and Cu, as demonstrated by the correlation coefficients as follows: Control ($r = 0.51$, $p < 0.05$), Group 2 ($r = 0.36$, $p = 0.09$), Group 3 ($r = 0.76$, $p \leq 0.05$), Group 4 ($r = 0.54$, $p \leq 0.05$), overall data ($r = 0.51$, $p \leq 0.05$).

Finally, the metals accumulation data for the spleen are presented in Tables A-25 through A-27 in the Appendix. Metals analysis was performed on the spleens of the early arriving rats only; therefore, it was not known if the concentration differed between these and the late arriving rats. Comparison of the data indicates that, at least for Group 4, the spleen accumulated considerably more Cd than did the muscle, lung or the heart. As with the other organs, the concentration of Cd in the spleen followed a dose-response relationship with ingested Cd. Data were not collected at 0, 3, 18 or 21 months; however, at all other times, and for the overall study as a whole, the concentrations of Cd in the spleens of Group 4 rats were significantly higher than those of all other groups (Table A-25).

Again, in a situation similar to that for the organs other than liver and kidney, the accumulation of Cd in the spleen had no effect on the accumulation of either Zn or Cu in this organ (Tables A-26 and A-27, respectively). Moreover, the correlation coefficients calculated for 0-24 months showed that there were no significant relationships between the concentrations of any two metals in the spleen.

ORGAN WEIGHTS

Tables A-28 through A-32 in the Appendix summarize the data on the total wet weight of the intestine, lung, heart, spleen, and liver. As shown in Table A-3, it was found that the early and late arriving rats differed significantly in organ weights as follows: intestine (6-21 months, overall), lung (3, 6, 18, 21 months, overall), liver (9 months, overall). Since the spleens of only the early rats, and the hearts of only the late rats were measured, it was not known if the two sets of animals differed significantly in the weights of these two organs. In order to present these data in the least confusing manner, the group mean organ weights shown in Tables A-28 through A-32 were calculated by treating the early and late arriving rats as one homogenous group. Thus, at each sacrifice, each value represents the mean ± standard deviation (S.D.) of organ weights for 6 to 8 rats per group.

After adjusting for differences between the early and late arriving rats, it was determined that there were no significant differences in organ weights between any of the dosage groups at any time. Thus, Cd dosing had no effect on the total weights of the liver, lung, intestine, spleen or heart, respectively.

Since both organ weight and rat body weight change as a function of age, the organ weights for each rat were recalculated as the percent of the total body weight. Tables A-33 through A-37 present the group mean percent values for the intestine, lung, heart, spleen, and liver, respectively. The early and late arriving rats differed significantly as follows: intestine (0, 15-24 months and overall), lung (0-6, 21, 24 months and overall), liver (3-9, 24 months and overall). Again, to present the data in the least confusing, most concise manner, the group mean percentage values in Tables A-33 to A-37 were calculated by treating the early and late arriving rats as one homogeneous group.

After adjusting for differences between the early and late arriving rats, it was found that there were no significant differences (at $p \leq 0.05$) between any dosage groups for the organ weights expressed as a percent of the body weight. Thus Cd dosing had no effect on the weight of the intestine, lung, heart, spleen, or liver as a function of the body mass.

Finally, a one gram sample of each organ was taken and the ratio of the wet:dry weight was calculated to provide a measure of the fluid content of the organ. Tables A-37 to A-42 summarize the group mean values of the wet/dry weights for the intestine, lung, heart, spleen and liver, respectively. Significant differences between the early and late arriving rats were as follows: intestine (0, 21 months), lung (3, 6 months, and overall) and liver (0, 12, 24 months and overall). As noted previously, data on the spleen were derived from the early rats while those on the heart were from the late rats. Except for the spleen and heart data, all other values in Tables A-37 to A-42 were calculated as the mean ± S.D. of 6 to 8 rats and the early and late arriving rats were treated as one homogeneous group.

After adjusting for differences between the early and late arriving rats, it was found that the only significant differences in organ wet/dry weight between the dosage groups was for the spleen (Table A-41). Although no significant group differences were found at any of the individual sacrifice points, Group 4 had a significantly higher spleen wet/dry weight than did the Controls for the data as a whole, irrespective of sacrifice point. Therefore, increased Cd exposure and increased organ Cd content were not associated with increased fluid content of the intestine, lung, heart or liver. On the other hand, Cd exposure at 50 μg/ml was associated with a slight, but significant increase in tissue fluid of the spleen.

ASSESSMENT OF CHANGES IN RENAL FUNCTION

Alterations in renal functioning have been assessed by observing changes in the activity of renal leucine aminopeptidase (LAP) and changes in the levels of urine parameters such as glucose, total protein, diurnal volume, pH, specific gravity and LAP. As indicated in the Materials and Methods section, urinalysis was performed on each rat just prior to sacrifice. Additionally, throughout the 24 months, urinalysis was performed every 4 to 8 weeks on the rats that were sacrificed at the end of the study. Data from the sacrificed rats have been used to determine the relationship between levels of renal Cd and changes in urine parameters. Graphical presentation of the urinalysis data has been limited to those values obtained from the periodic testing of the rats sacrificed at 24 months and does not include urinalysis data from the rats sacrificed at 3 through 21 months. This decision was made since the number of animals tested at any one time was small and there usually was a large interindividual variation. Thus, it was felt that the data were more accurately portrayed by using test values that represented a series of repeated measures from the same individual rats.

Renal Leucine Aminopeptidase

Figure 4-17 presents the data from the sacrificed rats on activity of the enzyme LAP in the renal cortex. The data are expressed as Sigma units/g cortex. A Sigma unit is the amount of enzyme that will release 1 mole (143 g) of β-naphthylamine from the substrate per hour, at 37°C and pH of 7.1. There are no data for 0 and 3 months because the tissue used for the enzyme assay was

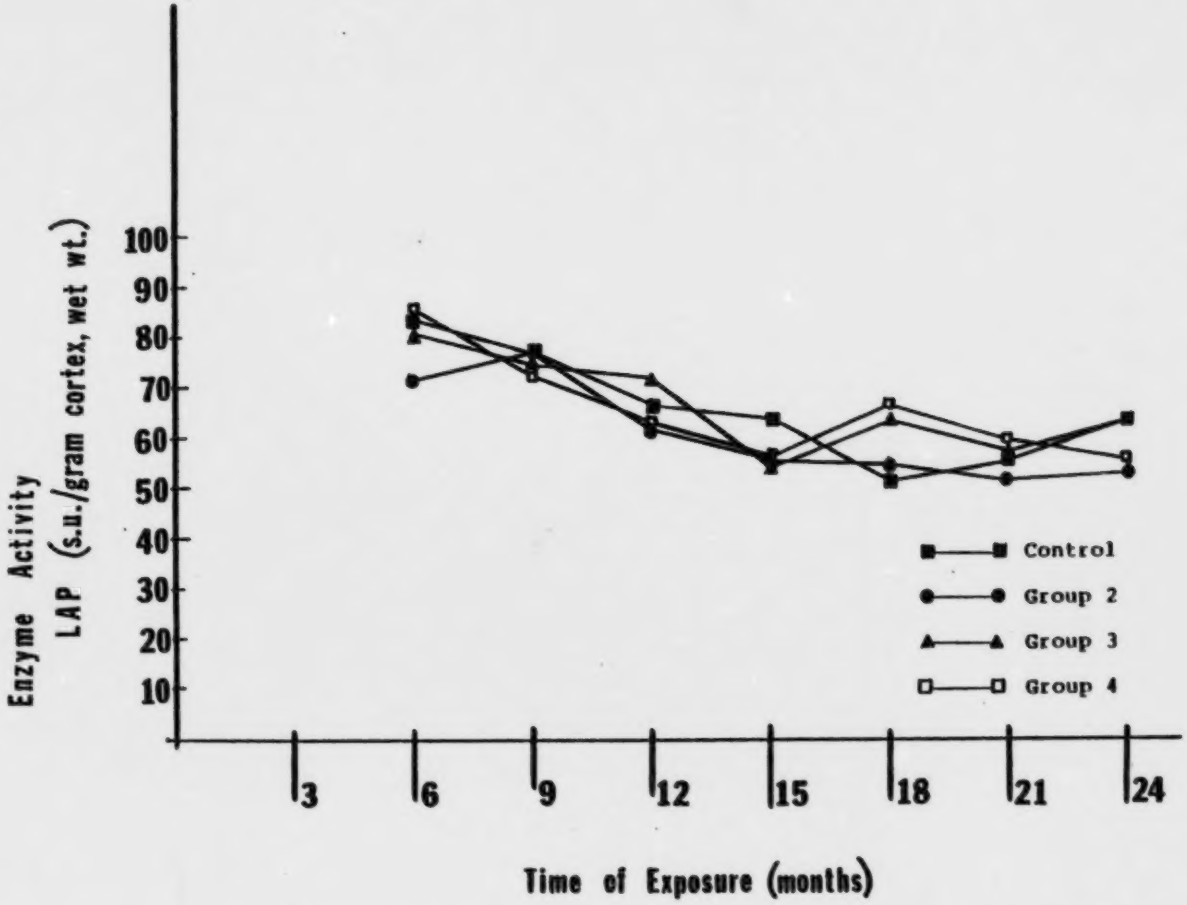


Figure 4-17. Mean Concentration of Renal Leucine Aminopeptidase, LAP (Sigma Units, S.U./g cortex, wet weight) as a Function of Age and Cadmium Exposure

processed the day of the sacrifice and then frozen at -20°C for the assay to be run at a later date. Samples handled in this manner rapidly lost their enzyme activity and were ruined. Starting at 6 months, all enzyme assays were run on fresh samples, within hours of the rat's death.

Early and late arriving rats differed significantly in their levels of renal LAP at 9, 18, 21 and 24 months, but not overall for the study as a whole (Table A-3). After adjusting for this difference, it was found that with the exception of 18 months, there were no significant differences between any exposure groups in their levels of enzyme activity. At 18 months, Group 4 had significantly higher levels of LAP than did the Controls ($p = 0.055$).

Irrespective of exposure group, there appeared to be an effect of age on the levels of renal LAP. Thus, there was a trend toward decreasing enzyme activity with age. From 6 to at least 15 months, there was a noticeable decrease in enzyme activity in all groups. Between 15 and 18 months, the levels in Groups 3 and 4 rose, while those in Group 2 and the Controls continued to decline. From 18 to 24 months, the levels in Groups 2 and 4 decreased while the levels in Group 3 and the Controls increased; however, there was no significant difference between the two groups.

Correlation coefficients relating both age and renal Cd concentration (µg Cd/g dry cortex) to the levels of renal LAP were tabulated for 6-15 months as follows:

	Control	Group 2	Group 3	Group 4	Overall
Renal Cd:Renal LAP	-0.14	-0.52*	-0.27	-0.38*	-0.09
Age:Renal LAP	-0.44*	-0.38*	-0.42*	-0.59*	-0.39*
Age:Renal LAP[a]	-0.45*	-0.36*	-0.33*	-0.46*	-0.39*

*Significant correlation coefficient at $p \leq 0.05$.

[a] Correlation coefficients calculated for 6-24 months.

See Table 4-1 for calculation of the number of rats used for determining correlation coefficients.

Since the concentration of renal Cd decreased after 15 months, the correlation coefficients were calculated for 6 to 15 months. For comparison, the correlation of age with LAP also was calculated for 6 to 24 months. As indicated, the relationship between age and enzyme levels appeared to be stronger than that between renal Cd and enzyme levels. Since renal Cd levels correlate positively with age up to 15 months, one cannot examine the correlation of renal Cd with enzyme activity without also looking at the correlation of age with enzyme activity.

Urine Leucine Aminopeptidase

Throughout the study, the early and late arriving rats differed significantly in the amounts of LAP eliminated in the urine. Since the early rats consistently excreted about twice as much enzyme as the late rats, it was felt that the data for these two sets of animals should be displayed separately. Thus, Figure 4-18A and -B present the patterns of urine LAP excretion for the early and late arriving rats, respectively. In each graph, data points represent the mean value of four rats within a particular exposure group.

After adjusting for differences between the early and late rats, it was determined that there were no significant differences in enzyme excretion between any of the groups at any time. Often there were substantial variations in enzyme excretion among the animals within each dosage group (Tables A-43 and A-44). It is possible that significant differences between the dosage groups were not detected as a result of this large variance in combination with the small sample size and close means.

Figures 4-18A and -B indicate an age-related trend in the excretion of LAP that was similar for all dosage groups. There was an initial increase in enzyme excretion in the young rats, up to about 25 to 35 weeks, followed by a gradual decrease through the end of the study. This long period of decreasing urine enzyme activity was similar to that seen for the renal enzyme levels (Fig. 4-17). Since there were no renal LAP data prior to 6 months, it cannot be determined if the initial rise in urine enzyme was coincident with high renal levels of enzyme in the very young rats.

Correlation coefficients calculated from 0 to 15 months showed that there was no relationship between the concentrations of Cd in the kidney cortex and the levels of LAP excreted in the urine. In contrast, there was a significant relationship between increasing age and decreasing excretion of LAP. Correlation coefficients relating both age and levels of renal LAP with daily excretion of urine LAP for 0-24 months were tabulated as follows:

	Control	Group 2	Group 3	Group 4	Overall
Age:Urine LAP	-0.44*	-0.49*	-0.30*	-0.34*	-0.24*
Renal LAP:Urine LAP	-0.17	0.25	0.20	0.21	0.21*
Age:Urine LAP[a]	-0.37*	-0.50*	-0.13	-0.02	-0.24

*Significant correlation coefficient at $p \leq 0.05$.

[a] Correlation coefficients calculated for 0-15 months.

See Table 4-1 for calculation of the number of rats used for determining correlation coefficients.

In addition to the age effect, there was a weak, but significant, direct relationship between renal and urine levels of enzyme activity. As indicated by the correlation coefficients, this relationship was evident only when the data as a whole, irrespective of group, were examined.

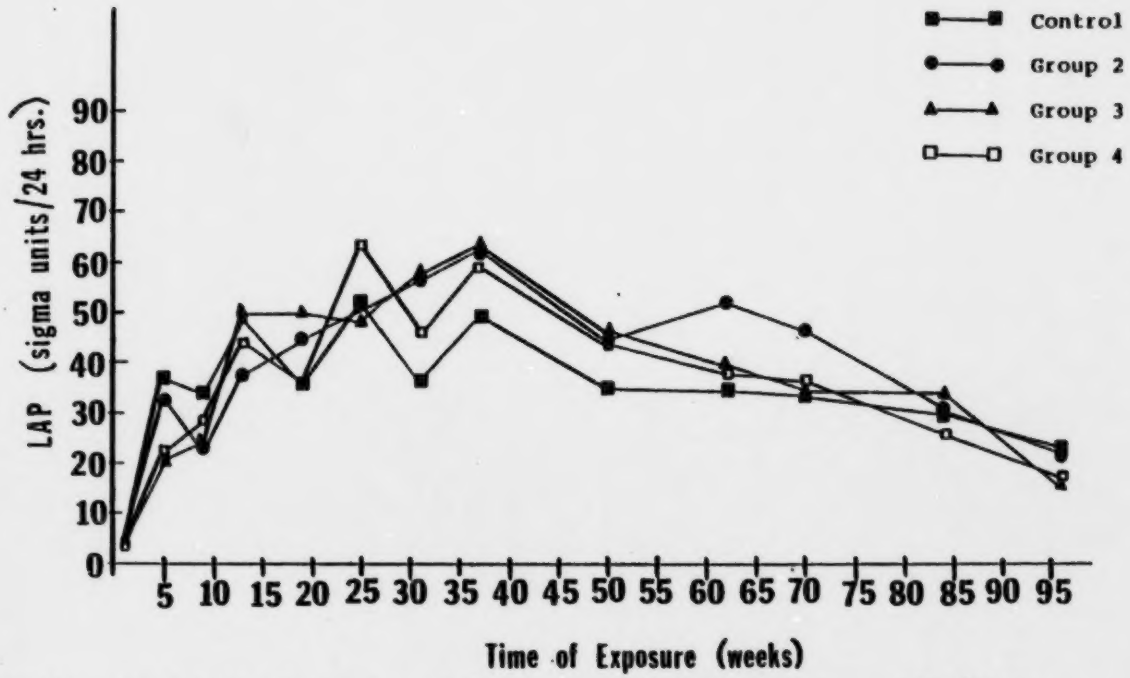


Figure 4-18A. Mean Daily Excretion of Urine Leucine Aminopeptidase, LAP (Sigma units/24 hours) as a Function of Age and Cadmium Exposure in Early Arriving Rats

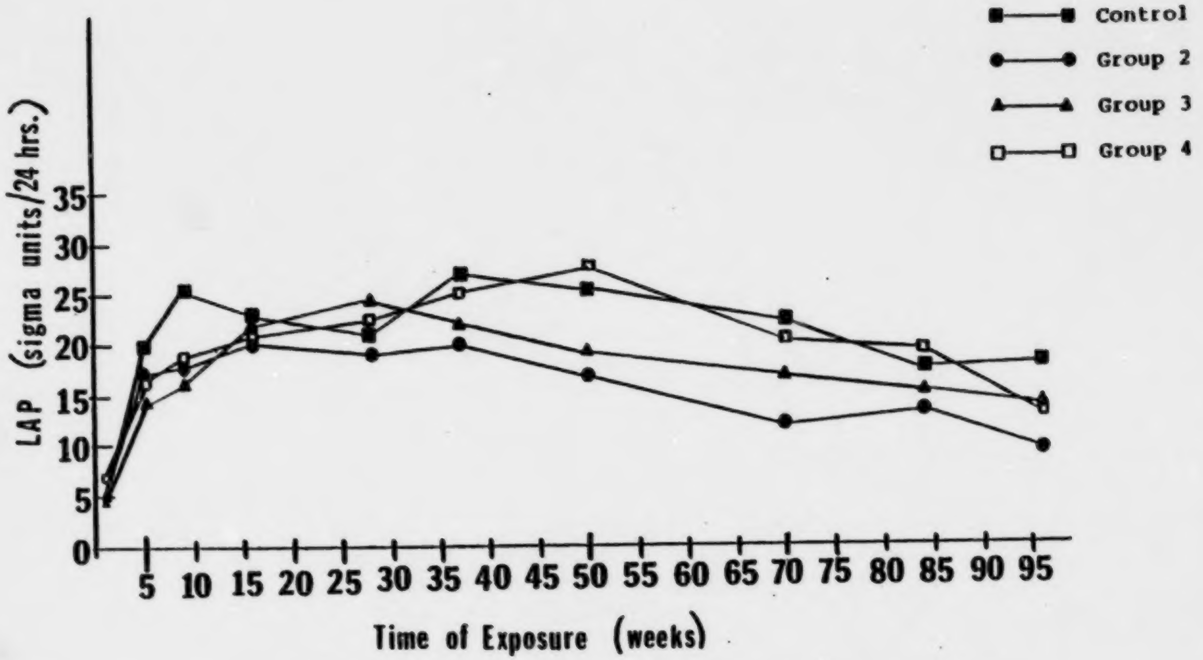


Figure 4-18B. Mean Daily Excretion of Urine Leucine Aminopeptidase, LAP (Sigma units/24 hours) as a Function of Age and Cadmium Exposure in Late Arriving Rats

Urine Volume

There were significant differences in daily urine volumes between the early and late arriving rats at 25, 62 weeks and overall. Figure 4-19 depicts the pattern of urine volumes voided daily by the different exposure groups. The data have been divided into early and late arriving rats, so that each point represents the mean value of 4 rats.

Analyses of variance of these data were conducted separately for the early versus late arriving rats, and the results have been presented in Tables A-45 and A-46 of the Appendix. As indicated in Table A-45, when the data for the early rats were examined for the study as a whole, Group 4 had significantly smaller urine volumes than did all other groups. Also, there were no significant difference in volumes among the Controls and Groups 2 and 3. Overall, for the late arriving rats (Table A-46), Groups 3 and 4 had significantly smaller urine volumes than did the Controls or Group 2. Unlike the early rats, there was no significant difference in volumes between Groups 3 and 4. It was expected that the high dose rats would have smaller urine volumes because they drank significantly smaller volumes of water than did the Controls and low dose rats (Figure 4-3).

As indicated in Figure 4-19, diurnal urine volumes appear to change with age. During the first 10 to 15 weeks, there was a significant increase in urine volumes in both early and late rats of the Controls and Group 2 and in the early rats of Group 3. Then through 50 weeks, volumes in these groups remained stable or decreased. In the early rats, from 65 weeks to the end of the study, there was a definite trend toward increased urine volumes in all groups. In contrast, urine volumes in the late rats tended to fluctuate after 65 weeks and showed no clear cut trend towards increasing. Unlike the other groups, Group 4 urine volumes remained small and stable throughout most of the study. There was, however, a definite trend in Group 4 toward increased urine volumes late in the study. Urine volumes increased in Group 4, both early and late rats, after week 65, with a more noticeable rise in the late rats.

Correlation coefficients relating age and renal Cd concentrations (μg Cd/g dry cortex) to daily urine volumes for 0-15 months are as follows:

	Control	Group 2	Group 3	Group 4	Overall
Age:Urine Volume	-0.51*	-0.39*	-0.33*	-0.53*	-0.057
Renal Cd:Urine Vol.	0.07	-0.12	-0.02	-0.51*	-0.42*
Age:Urine Volume[a]	-0.05	-0.10	0.09	0.26*	0.06

*Significant correlation coefficient at $p \leq 0.05$.

[a] Correlation coefficient calculated for 0-24 months.

See Table 4-1 for calculation of the number of rats used for determining correlation coefficients.

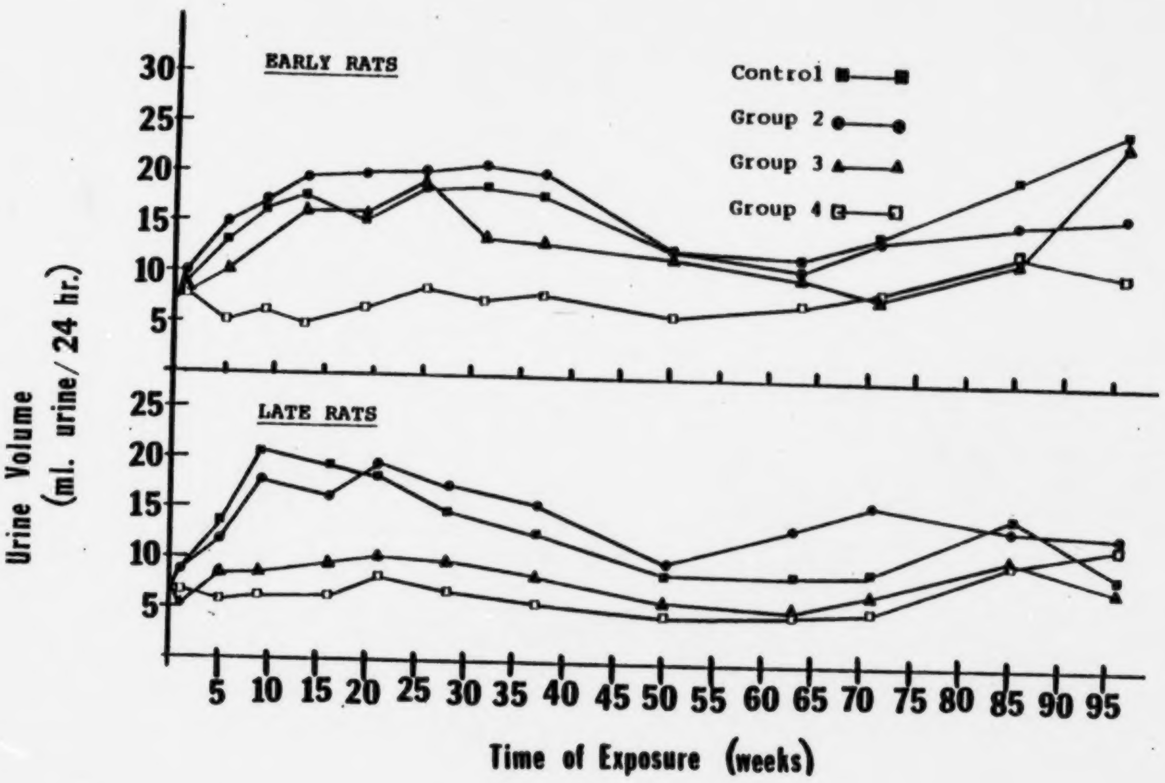


Figure 4-19. Mean Daily Urine Volume (ml/24 hours) as a Function of Age and Cadmium Exposure

In the younger rats, 0-15 months, there was a significant relationship between increasing age and decreasing urine volume for each of the test groups; however, there was no correlation when all the data were examined as a whole, regardless of group. As expected, there was a significant correlation of high renal Cd with low urine volumes, as seen for Group 4 and the data as a whole. This latter relationship probably reflects the fact that water consumption, and therefore urine volume, varied inversely with dose.

It was interesting that when age and urine volume were correlated for the whole study period, 0-24 months, the correlation coefficients were quite different from those calculated for 0-15 months. As indicated, there was no significant relationship between age and urine volume except in Group 4. Although the relationship was weak, it seemed to indicate that old age, in combination with high Cd exposure, was significantly correlated with increased urine volumes. In contrast, old age alone or in combination with low Cd exposure had no effect on urine volume.

Urine Total Protein

Throughout the whole study, the early arriving rats excreted significantly higher amounts of total protein (TP) than did the late arriving rats ($p \leq 0.05$). Since the early rats excreted roughly twice as much protein as the late rats, the data for each set of rats have been graphed separately (Figure 4-20A and -B).

After adjustment for differences between early and late rats, analysis of variance showed that there were no significant differences in TP between any of the groups at any time. Bartholomew's test for ordered means also showed that the differences were not significant ($p \leq 0.05$). As noted previously with other tissue and urine parameters, there was a large variation in protein levels within each test group at many of the test points. Group means and standard deviations for TP have been summarized for the early and late rats in Tables A-47 and A-48, respectively. The group variances are often extremely large because at any sampling point, a variable number of rats had proteinuria. Thus, starting with week 37 in the early rats, and week 62 in the late rats, varying percentages of individuals within each group had high TP (i.e., 100+ mg/24 hours) as compared to low TP (i.e., 20-60 mg/24 hours). Significant differences in TP between the groups probably were not detected as a result of this large variance in combination with small sample size, and close means.

Figures 4-20A and -B show that there was a significant effect of age on levels of TP. As expected, older animals lost considerably larger quantities of protein compared to younger individuals. Generally, in both the early and late rats, week 50 appeared to be the point at which the rate of protein excretion started to increase more noticeably.

Although significant group differences were not found, Figure 4-20A indicates that in the early rats, there was a trend, starting at week 30, for TP in Group 4 to be higher than that in the Controls. Also, starting at week 20, Group 3 protein levels tended to be higher than Controls. Group 2 levels were very close to those of the Controls up to week 60, but thereafter were noticeably lower. A similar situation was seen in the late rats (Fig. 4-20B).

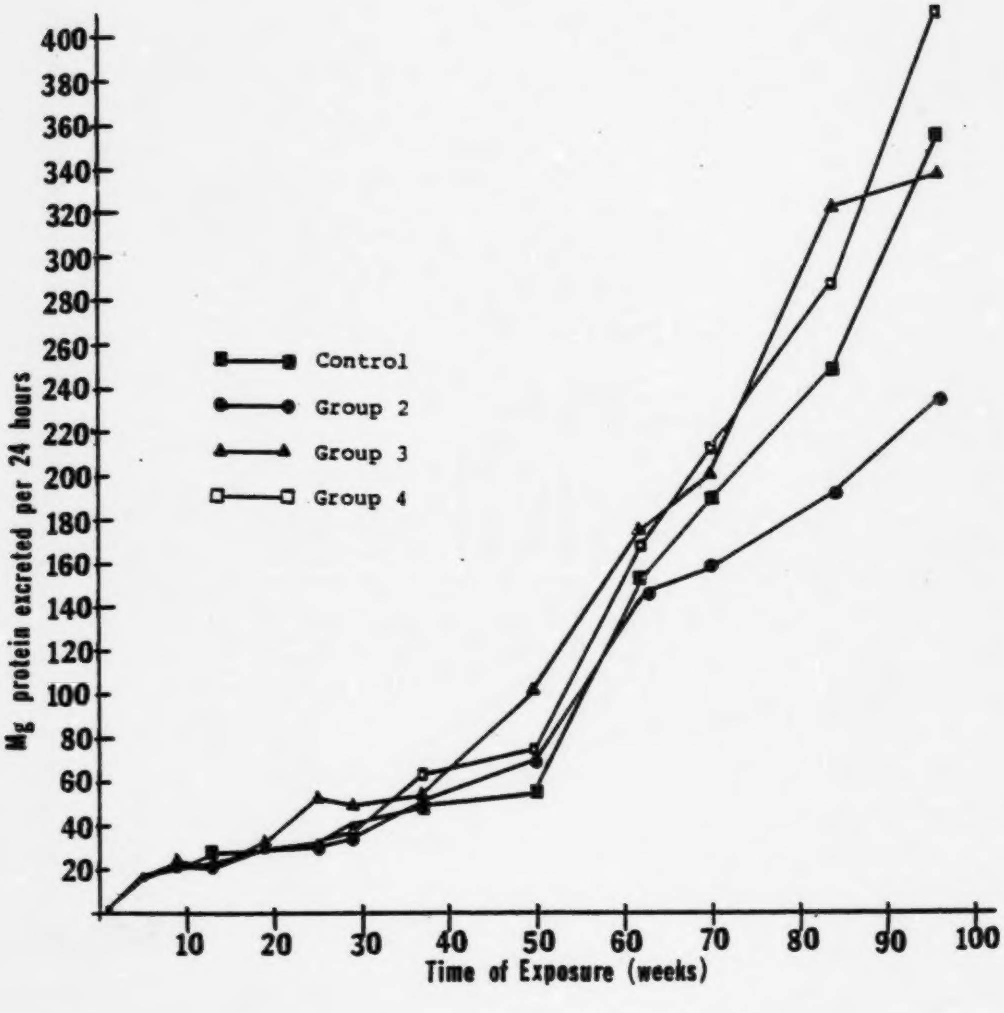


Figure 4-20A. Mean Daily Excretion of Urine Total Protein (mg/24 hours) in Early Arriving Rats as a Function of Age and Exposure to Cadmium

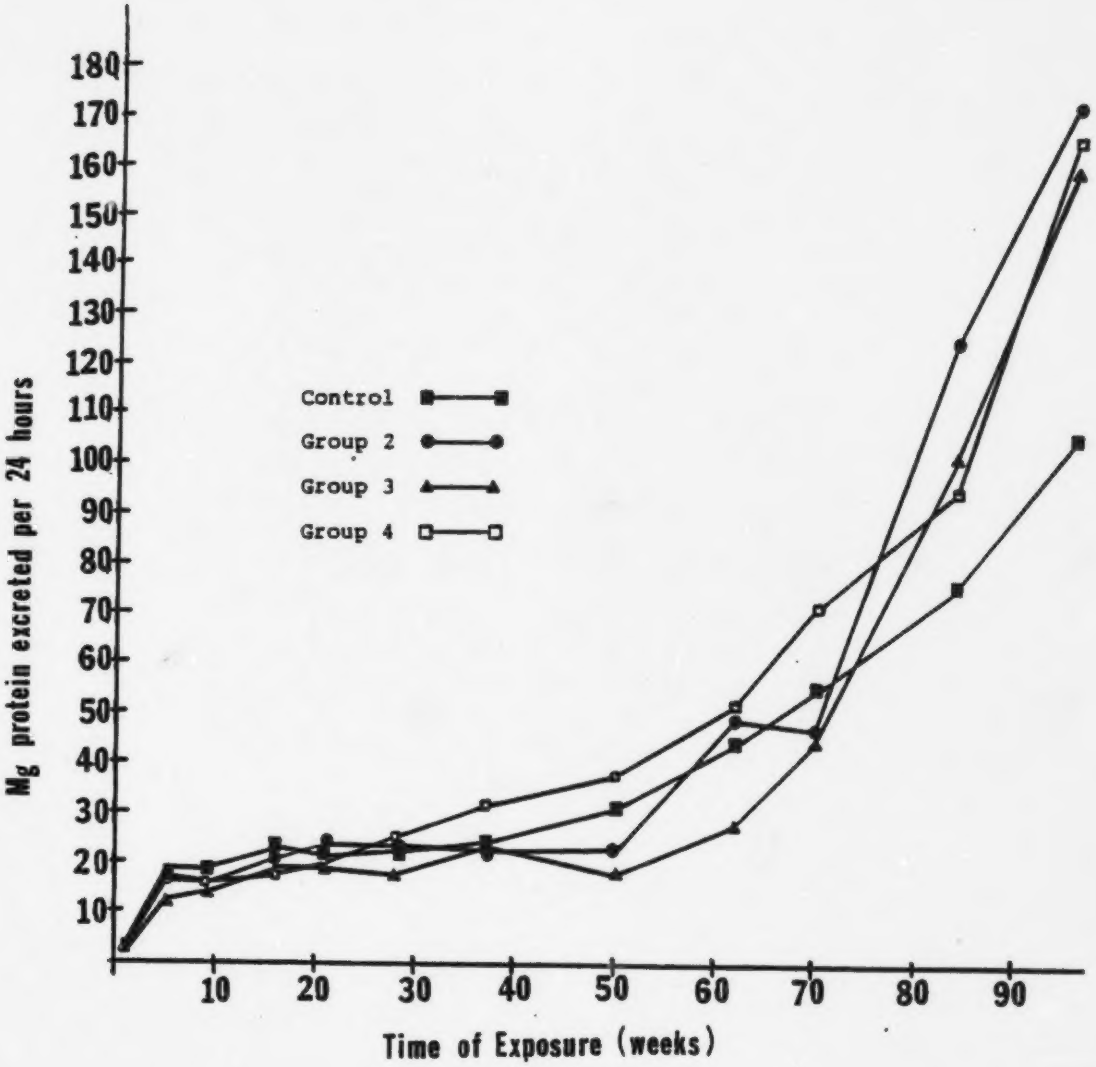


Figure 4-20B. Mean Daily Excretion of Urine Total Protein (mg/24 hours) in Late Arriving Rats as a Function of Age and Exposure to Cadmium

Also starting around week 30, Group 4 protein levels were noticeably higher than Control values. Groups 2 and 3 TP tended to be near or below Control levels until after week 70. Thereafter, the protein levels in these two groups were considerably higher than those in the Controls and actually were very close to the levels found in Group 4.

Correlation coefficients relating both age and renal Cd concentrations (μg Cd/g dry cortex) to the levels of daily TP for 0-15 months are summarized as follows:

	Control	Group 2	Group 3	Group 4	Overall
Age:TP	0.48*	0.56*	0.52*	0.61*	0.64*
Renal Cd:TP	0.28	0.71*	0.30	0.34*	0.04
Age:TP[a]	0.58*	0.60*	0.64*	0.69*	0.64*

*Significant correlation coefficient at $p \leq 0.05$.

[a] Correlation coefficient calculated for 0-24 months.

See Table 4-1 for calculation of the number of rats used for determining correlation coefficients.

Obviously, throughout the two years, there was a strong correlation between increasing age and proteinuria. Since the concentrations of renal Cd decreased considerably after 15 months, it was felt that the correlation of renal Cd with TP should not be calculated for data beyond that point as it would have yielded a correlation coefficient of questionable value. Unfortunately, the greatest increases in TP occurred after 15 months. Nevertheless, up to 15 months, there was a significant positive correlation of renal Cd with TP in Groups 2 and 4.

Urine Glucose

Unlike the excretion of total protein and LAP, the early and late rats did not differ significantly in their levels of glucose excretion. Figure 4-21 presents the data on daily levels of glucose lost in the urine. The graph was constructed by pooling the data from the early and late rats so that each point usually represents the mean value of 8 rats.

Using analysis of variance and Bartholomew's test for ordered means, no significant differences in urine glucose were found between any two groups at any time. However, Figure 4-21 does indicate certain trends between Cd exposure and glucose excretion. From 1-15 weeks and from 50 weeks to the end of the study, the mean levels of glucose excreted by the Controls were very stable. Also, in Group 2, despite considerable fluctuations prior to week 50, the mean levels of glucose excreted after this point were very stable and almost identical to those in the Controls. In Group 3, fluctuations in mean glucose levels generally paralleled those of the Controls up to week 60, but

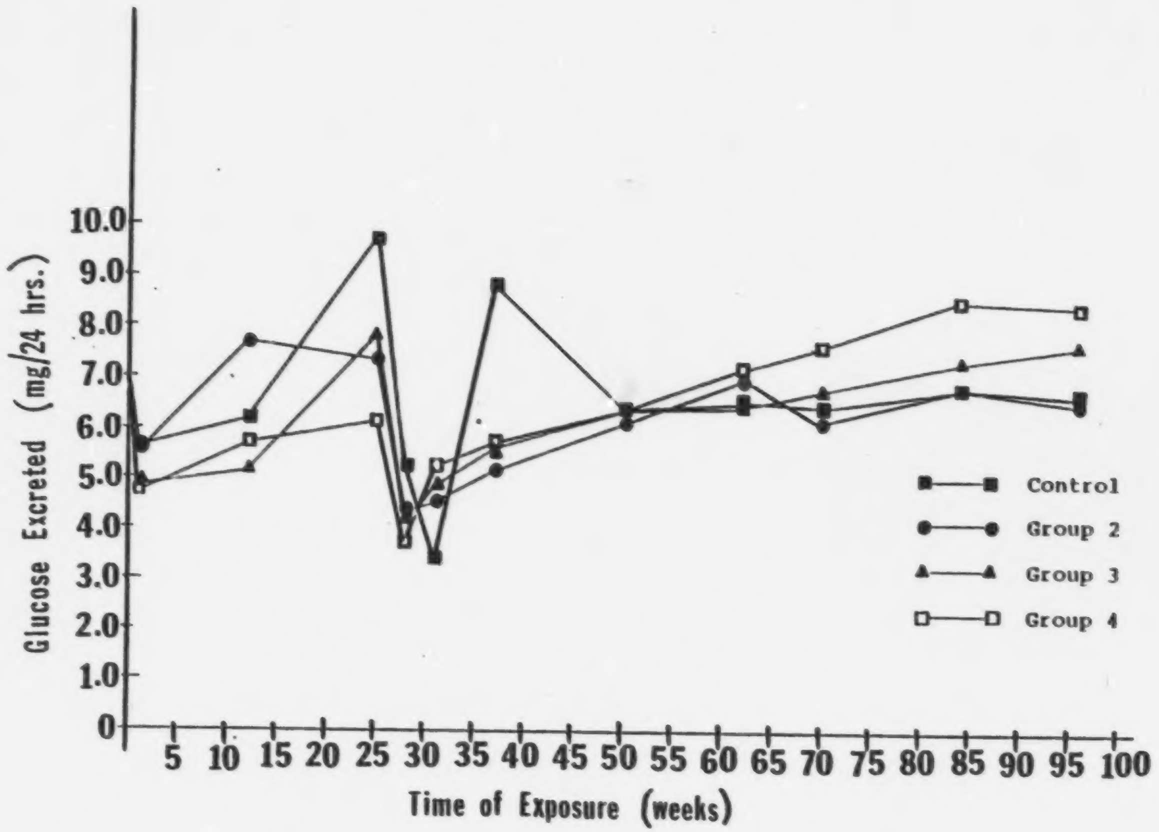


Figure 4-21. Mean Daily Excretion of Glucose (mg/24 hours) in the Urine as a Function of Age and Exposure to Cadmium

thereafter the levels were noticeably elevated above Controls. Finally, in Group 4, starting around week 30, there was a steady increase in the rate of glucose excretion which continued almost to the end of the study. After week 50, the levels of glucose from Group 4 were noticeably higher than those from the Controls. These trends were reflected in the values of the correlation coefficients. Coefficients calculated for age and renal Cd (as μg Cd/g dry cortex) versus daily excretion of glucose (mg/day) for 0-15 months were summarized as follows:

	Control	Group 2	Group 3	Group 4	Overall
Age:Glucose	0.07	-0.15	0.08	0.03	0.24*
Renal Cd:Glucose	0.10	0.06	0.13	-0.16	-0.07
Age:Glucose[a]	0.20	0.08	0.30*	0.46*	0.24*

*Significant correlation coefficient at $p \leq 0.05$.

[a] Correlation coefficient calculated for 0-24 months.

See Table 4-1 for calculation of the number of rats used for determining correlation coefficients.

As indicated, in younger rats, up to 15 months, there was a weak but significant relationship between increasing age and increasing excretion of glucose. Although no such relationship was evident on a group basis, it was seen when all the data were considered as a whole, regardless of group. For this same time period, there was no relationship between renal Cd concentration and glucose excretion. In contrast, when the correlation coefficients were calculated for 0-24 months, a significant correlation with age appeared in Groups 3 and 4, but not in Control or Group 2. Apparently, the combination of higher Cd exposure with advanced age was related to increased glucose excretion.

Urine pH and Specific Gravity

Group mean values for the pH and specific gravity of urine are presented in Tables A-49A, -B and A-50A, -B, respectively. For each rat, both measurements were made on 3 separate urine samples that were collected over three consecutive 24 hour periods. The three values for each rat were averaged and these average values were used in the analysis of variance and correlation coefficient calculations. The data shown in Tables A-49 and A-50 represent the group means of these averaged values.

As indicated in Tables A-49A and -B, there was a consistent trend throughout the study for Group 2 to have the highest pH values, although they were never significantly higher than the Controls ($p \leq 0.05$). The pH then varied inversely with dose for Groups 3 and 4. Overall, in the late rats, the pH values for Groups 3 and 4 were both significantly lower ($p \leq 0.05$) than the pH values for Group 2. There was no significant difference between the Controls and Group 2 or between Groups 3 and 4. The pH values of the early rats were less affected by Cd exposure. Thus, overall, the pH values of the Group 4 early rats were significantly lower than the pH values of Group 2, but at p = 0.10.

The correlation coefficients relating both age and renal Cd concentrations (μg Cd/g dry cortex) to urine pH for 0-15 months are as follows:

	Control	Group 2	Group 3	Group 4	Overall
Age:Urine pH	-0.46*	-0.54*	-0.43*	-0.52*	-0.59*
Renal Cd:Urine pH	0.03	-0.70*	-0.38*	-0.57*	-0.34*
Age:Urine pH[a]	-0.62*	-0.69*	-0.62*	-0.62*	-0.59*

*Significant correlation coefficient at $p \le 0.05$.

[a]Correlation coefficient calculated for 0-24 months.

See Table 4-1 for calculation of the number of rats used for determining correlation coefficients.

As indicated, there was a significant, strong correlation between increasing age and more acidic urine for all groups over both 0-15 and 0-24 months. Additionally, there was a strong, significant correlation between higher concentrations of renal Cd and more acidic urine; however, there also was an age component involved since the concentration of renal Cd increased with age up to 15 months.

Tables A-50A and -B show that, throughout the study, in both the early and late rats, there was a dose-response relationship between Cd exposure and urine specific gravity. This was expected since the high dosed rats produced significantly smaller volumes of urine. For the individual sampling points, Group 4 usually had significantly higher specific gravity than the Controls or Group 2. In both the early and late rats, for the overall study, Group 4 was significantly higher than all other groups and, in addition, Group 3 was significantly higher than the Controls or Group 2. There was no significant difference between the Controls and Group 2.

Examination of the tabulated data also showed that there may have been an age related trend to the changes in specific gravity. For each group, the specific gravity fluctuated but generally increased to reach a maximum value around week 50 to 62. Thereafter, the values decreased through the end of the study. These changes were reflected in the correlation coefficients for 0-15 months, as indicated below.

	Control	Group 2	Group 3	Group 4	Overall
Age:Urine Specific Gravity	0.52*	0.50*	0.31*	0.64*	0.07

Renal Cd Concentration: Urine Specific Gravity	-0.01	0.19	-0.02	0.48*	0.63*
Age:Urine Specific Gravity[a]	0.20	0.16	0.14	-0.23[1]	0.07

*Correlation coefficient significant at $p \leq 0.05$.

[1] Correlation coefficient significant at $p = 0.072$.

[a] Correlation coefficient calculated for 0-24 months.

See Table 4-1 for calculation of the number of rats used for determining correlation coefficients.

As expected, there was a strong correlation between age and specific gravity for each of the groups up to 15 months. In contrast, of the individual groups, only Group 4 showed a significant correlation between renal Cd concentration (μg Cd/g dry cortex) and specific gravity; however, the correlation was weaker than that seen between age and specific gravity. In the younger rats, the data overall, regardless of group, indicated a different outcome. Thus, when individual groups were ignored, age had no effect on urine specific gravity while renal Cd levels had a significant effect.

The correlation coefficients calculated for 0-24 months show that when the data from the old rats was added, there no longer was a significant association between age and specific gravity for the Controls or the two low exposure groups. In contrast, for Group 4, there was a weak association between increasing age and decreasing specific gravity that was significant at $p = 0.072$.

Referring back to the graphs and correlation coefficients dealing with urine volume, it was seen that the specific gravity data corresponded to the volume data. In each of the groups, up to 15 months, increased age correlated positively to smaller volumes of more concentrated urine. Also, in the younger rats, higher concentrations of renal Cd were associated with smaller volumes of more concentrated urine. When data from the old rats were added, the combination of increasing age and high Cd exposure correlated with larger volumes of more dilute urine.

ASSESSMENT OF CHANGES IN THE STRUCTURE OF THE KIDNEY

Changes in the structure of the kidney have been examined based on the following variables: organ wet weight, organ weight as a percentage of body weight, ratio of wet/dry weight, and tissue morphology.

Kidney Weight

For the study as a whole, regardless of individual sacrifice points, the early and late arriving rats differed significantly in the total wet weight of the kidneys (Table A-3). Additionally, the left kidney weights were significantly different ($p \leq 0.05$) at 12, 18, and 24 months, while the right kidney weights were significantly different at 12, 15 and 18 months.

After adjusting for differences between the early and late arriving rats, no significant differences in kidney weights were found between any of the groups at any time other than 18 months. At this particular time, Group 4 kidneys weighed significantly more than Group 2 kidneys. Therefore, a significant dose-response relationship was not found between Cd exposure and kidney wet weight.

Figure 4-22 presents the data on group mean kidney weight as a function of age and exposure to Cd. Each point represents the group mean weight of both right and left kidneys of 6 to 8 rats. Although the early and late rats differed significantly in kidney weights, the actual differences were small and consistent. Therefore, the data have been pooled to construct a single graph. As indicated in the figure, the most rapid increase in kidney weight occurred in all groups from 0 to 3 months. The weights stabilized from 3 to 12 months and then increased again through the end of the study. Although the weights were not significantly different, there was a trend for Group 4 kidneys to be consistently heavier than Control kidneys from 6 months to the end of the study.

Since both the kidney and rat body weights changed as a function of age, the kidney weights were recalculated as a percent of the total body weight. As indicated in Table A-3, the early and late rats differed significantly in this variable (for both the right and left kidneys) at four different sacrifice points and for the study as a whole, regardless of sacrifice point. After adjusting for these differences, it was found that the Group 4 kidneys, both left and right, accounted for a significantly higher percentage of the total body mass as compared to the Controls ($p \leq 0.05$). The data relating kidney weight to body weight are summarized in Tables A-51A and A-51B for the left and right kidneys, respectively. As indicated in these tables, the weight of the kidney relative to the weight of the body varied directly with dose throughout most of the study. In addition to the significant difference between Group 4 and the Controls (at $p \leq 0.05$), Group 4 left kidney values also were significantly higher than Groups 2 and 3 (at $p = 0.10$) for the overall study as a whole.

Figure 4-23 shows the mean kidney weight as a percentage of the body weight. For graphical purposes, the early and late rats have been treated as one homogeneous group and the data from the left and right kidneys have been pooled. Thus, each point represents the group mean of both left and right kidneys for 6 to 8 rats. As indicated, the weight of the kidney relative to body mass changed as a function of age. The most rapid decline in the value of this parameter occurred in all groups during the first three months. The values continued to decrease at a slower rate, through 9 months, then stabilized until 18 months, and finally increased again through 24 months. From 3 months to the end of the study, Group 4 values were consistently elevated above the Controls.

Ratio of Wet Weight to Dry Weight of Kidney Cortex

For each rat, the ratio of wet/dry weight was calculated for the cortex of the left kidney in order to provide a relative measure of the tissue fluid. With the exception of 0 and 9 months, there were no significant differences in

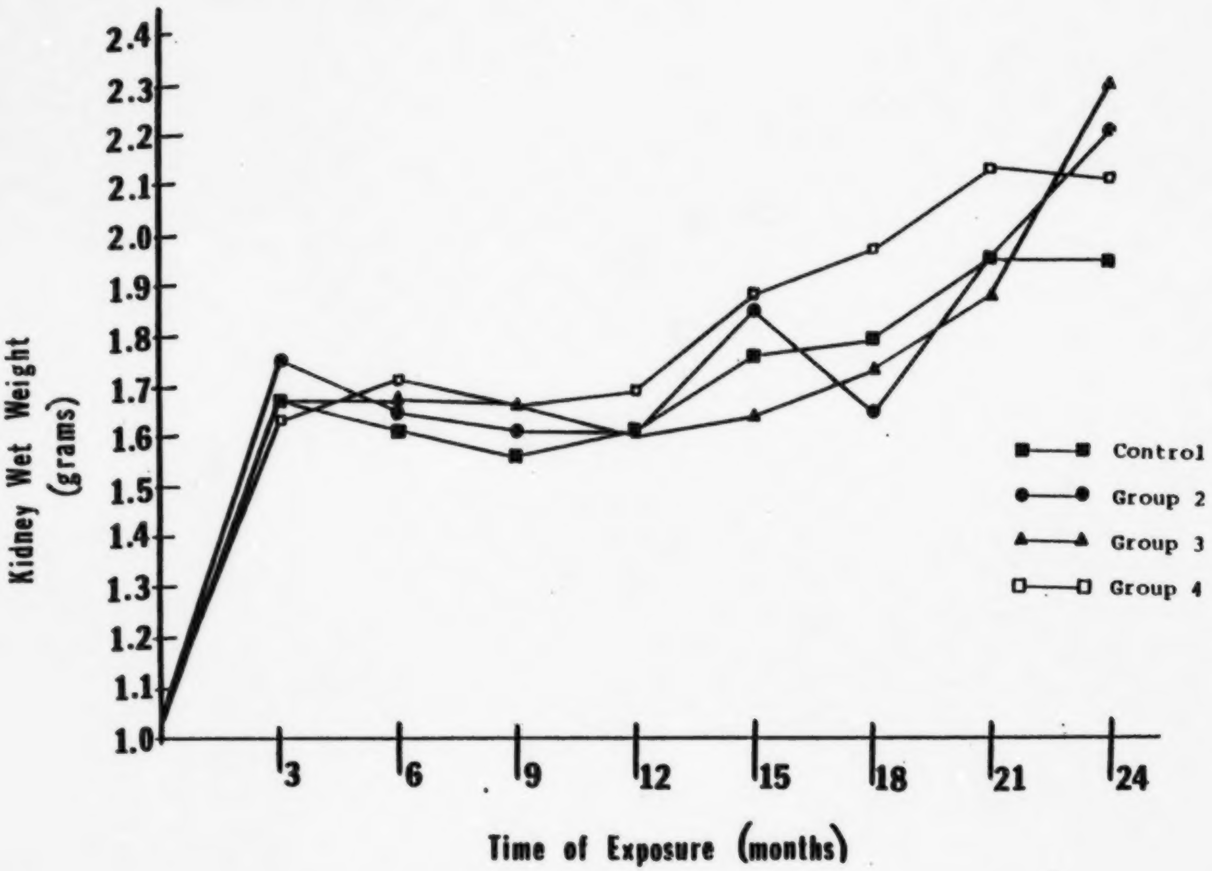


Figure 4-22. Mean Kidney Wet Weight (grams) as a Function of Age and Exposure to Cadmium

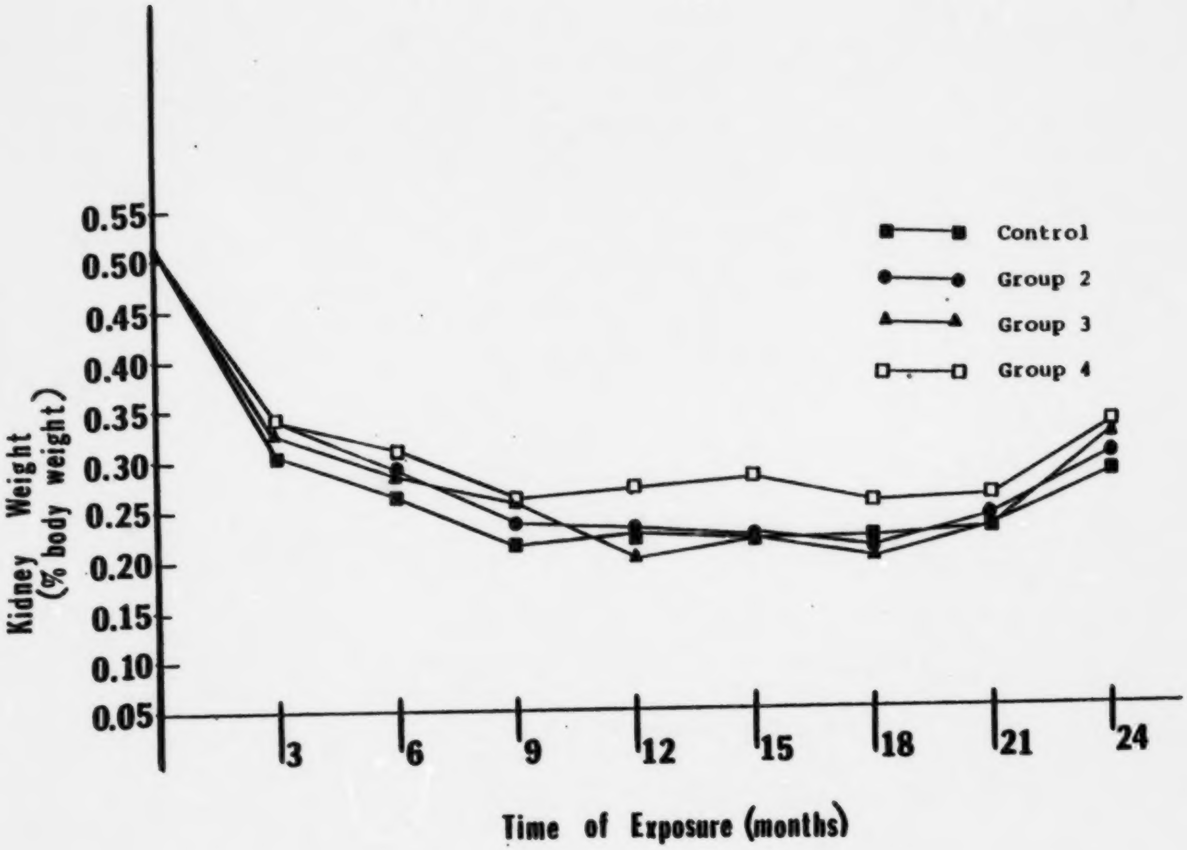


Figure 4-23. Mean Kidney Wet Weight as a Percent of the Total Body Weight as a Function of Age and Cadmium Exposure

this parameter between the early and late rats. Moreover, there were no significant differences between any of the groups at any time during the study.

As indicated in Figure 4-24, the volume of renal tissue fluid changed as a function of age. In this figure, each point represents the group mean value of 6 to 8 rats. There was an almost linear increase in tissue fluid with age from 0 to 18 months in all groups except the Control. From 18 months to the end of the study, there was a substantial decrease in the tissue fluid of all groups, but particularly in the Controls. Although significant differences were not detected between the groups, Figure 4-24 shows that throughout most of the study, the treatment groups contained more tissue fluid than did the Controls.

Tissue Morphology

Changes in the tissue structure of the kidney were assessed at 0, 9, 15 and 24 months of exposure. Tables A-52A and A-52B summarize the data concerning the size of the glomeruli, expressed as the diameter of Bowman's capsule. Because the profiles of the glomeruli ranged from circular to somewhat elliptical, the group mean values of both the long and the short diameters were calculated (Table A-52A). Additionally, at each time point, both dimensions of all glomeruli were averaged to yield a single mean diameter value for each group (Table A-52B).

As indicated in these tables, there was a definite age-related trend in glomerular size for all groups. For all groups, there was a significant increase ($p \leq 0.05$) in the size of the glomeruli with age. In contrast, the average size of the glomeruli was not significantly affected by Cd exposure ($p \leq 0.05$).

Although the mean glomerular size as a function of age was not significantly affected by Cd exposure, the frequency distribution of the average glomerular diameter appeared to vary with dosage. Figure 4-25 presents the group frequency distributions of the glomerular average diameter. There appeared to be a shift towards larger glomeruli in Group 4 as compared to the Controls. The Controls had a mode of 120-130 μm, while Group 4 had a mode of 130-140 μm. Also, the percentage of rats having average glomerular diameters greater than or equal to 120 μm was 75% in Group 4 (N = 23) as compared to 65% in the Controls (N = 23).

Morphometrics were used to measure the volume density (Vv), numerical density (Nv), and numerical profile density (Na) of the glomeruli in the outer cortex. As indicated in the Materials and Methods section, the volume density, measured by grid point counting, is a proportion representing the total volume of glomeruli per unit volume of cortex and, as such, does not measure the actual number of glomeruli.

Since the total number of glomeruli per kidney could not be counted, the glomerular numerical density was used to provide a relative measure of the change in the number of glomeruli as a function of both age and Cd dosing. Unlike Na, which is the number of glomerular profiles per unit area of a random kidney section, the numerical density is based on both the size distribution

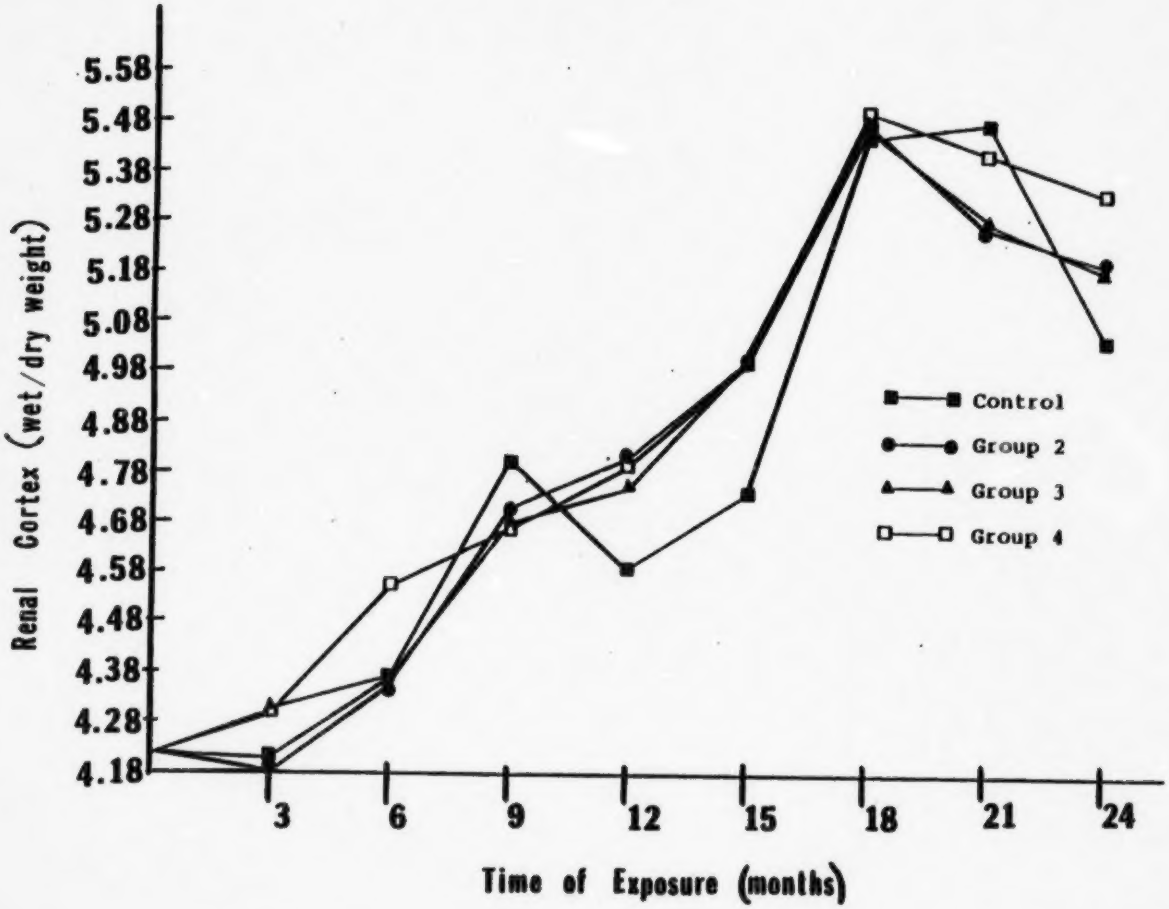
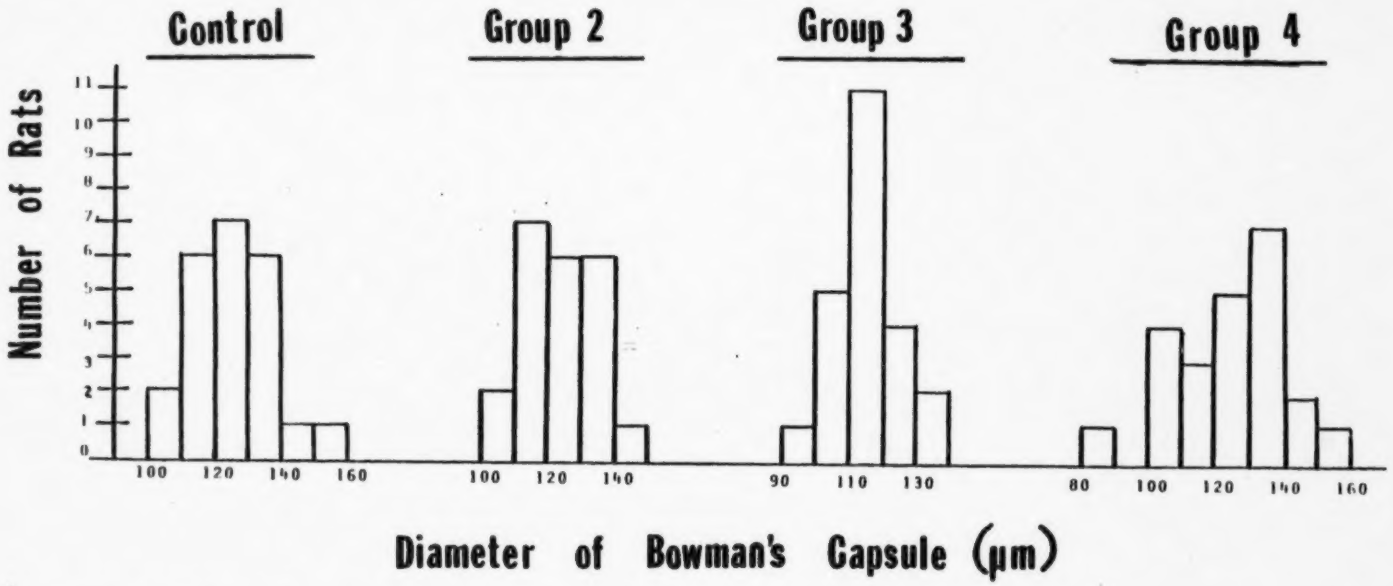


Figure 4-24. Kidney Cortex Wet/Dry Weight as a Function of Age and Cadmium Exposure



[1] Average diameter of each glomerulus was calculated as (long diameter + short diameter)/2. The mean value of 15 glomeruli per rat was used to construct each histogram.

Figure 4-25. Frequency Distribution of the Glomerular Average Diameter (µm)[1] as a Function of Exposure to Cadmium

and area profile density of the glomeruli. Because the sizes of the glomeruli vary within and between animals and also change as a function of age, it was important to calculate the numerical density. Group mean values for glomerular Vv, Na and Nv are presented in Tables A-53, A-54 and A-55, respectively.

Both the numerical profile density and the numerical density of the glomeruli decreased significantly with age ($p \leq 0.05$) for all groups. Because these two parameters relate the number of glomeruli to a unit area or volume of cortex, they show that the density of glomeruli decreased with age and this decrease started when the animal was relatively young (i.e., between 1 and 9 or 10 months of age). Cd exposure did not have a significant effect on either of these two parameters.

The glomerular volume density remained stable up to 15 months for Control, Groups 2 and 3 and up to 24 months for Group 4. Although the volume density decreased at 24 months for the Controls and two lower exposure groups, the change was not significant when compared to the baseline value. As indicated in Table A-53, the Group 4 volume density was significantly higher than that of Groups 2 and 3 at 24 months; however, Group 4 was never significantly different from the Controls.

Correlation coefficients relating both age and renal Cd concentrations (µg Cd/g dry weight of cortex) to glomerular volume and numerical densities are presented in Table 4-3.

In general, there was a significant correlation between increasing age and decreasing volume density, numerical profile density and numerical density of glomeruli. In contrast, there was no consistent significant correlation between renal Cd levels and any of these measures of renal structure. That renal Cd correlated positively with volume density for 0-24 months probably occurred because the Cd concentrations decreased significantly after 15 months. When the correlation coefficients were recalculated for 0-15 months, there was no relationship between renal Cd and glomerular volume density. Interestingly, of the four study groups, only Group 4 showed a significant relationship between age and decreasing numerical density of glomeruli.

An attempt was made to assess the changes with age in the amount of normal proximal tubular tissue. To accomplish this, the surface volume density of the tubular walls was determined for the outer cortex. This measurement was complicated by age-related changes in the kidney which caused increased tubular dilation, tubular hypertrophy and interstitial fibrosis. For this reason, random fields were sampled only in areas of the outer cortex having reasonably intact proximal tubules. Since the surface density was determined by counting the number of times tubular walls crossed the horizontal test line of the grid, counts were made only for those tubules that appeared normal. In view of these restrictions, the surface density of normal proximal tubules in the outer cortex was found to decrease with age in all groups. This trend was significant ($p \leq 0.05$); however, it was not affected by Cd exposure.

Finally, typical age-related changes in renal structure were assessed by ranking the degree of inflammation, interstitial fibrosis, tubular dilation, tubular hyperplasia, and thickening of the glomerular and proximal tubular

Table 4-3. Correlation Coefficients Relating Age and Renal Cd Levels to Glomerular Volume Density, Numerical Profile Density and Numerical Volume Density

0 - 24 Months	Control	Group 2	Group 3	Group 4	Overall
Age:Volume Density	-0.37[a]	-0.78*	-0.52*	0.24	-0.34*
Age:Numerical Profile Density	-0.26	-0.67*	-0.34	-0.40*	-0.56*
Age:Numerical Volume Density	0.13	0.19	0.24	-0.46*	-0.27*
Renal Cd:Volume Density	0.25	0.31	0.24	0.22	0.21*
Renal Cd:Numerical Profile Density	0.03	-0.22	0.11	-0.34	-0.02
Renal Cd:Numerical	-0.19	-0.55*	-0.09	-0.39[d]	-0.17

0-15 Months	Control	Group 2	Group 3	Group 4	Overall
Age:Volume Density	0.15	-0.33	-0.11	0.48[e]	0.07
Age:Numerical Profile Density	-0.24	-0.65*	-0.22	-0.67*	-0.70*
Age:Numerical Volume Density	-0.30	-0.42	-0.11	-0.73*	-0.66*
Renal Cd:Volume Density	0.21	-0.34	0.06	-0.04	-0.04
Renal Cd:Numerical Profile Density	-0.02	-0.72*	-0.24	-0.44[b]	-0.11
Renal Cd:Numerical Volume Density	-0.16	-0.50[a]	-0.22	-0.42	-0.10

*Correlation coefficient significant at $p \leq 0.05$.

[a] Correlation coefficient significant at $p = 0.084$.

[b] Correlation coefficient significant at $p = 0.097$.

[e] Correlation coefficient significant at $p = 0.060$.

At each sacrifice, data were collected on 7 to 8 rats per test group. Therefore, the correlation coefficients calculated for 0-15 months were based on 22 to 24 rats per group while those calculated for 0-24 months were based on 29-32 rats per group. These figures include the 8 rats sacrificed at the zero time point.

"Overall" correlations were calculated by combining all the renal data, irrespective of exposure group, for either 0-15 or 0-24 months. For 0-15 months, the correlation coefficients were based on the data for 64 to 72 rats. For 0-24 months, the coefficients were based on the data for 92 to 104 rats.

basement membranes. Each parameter was rated on a scale from 0 to 5. The severity of changes were ranked mild (0-2), moderate (2.5-3.5) or severe (4-5). The data for each parameter were analyzed in two ways. First, at each time point, the mean scores were calculated for each group (Table A-56). Second, the percentages of individuals within each group that had either mild, moderate or severe changes were determined (Table A-57).

Figures 4-26 through 4-28 show the progression of these age-related renal changes in 1, 15 and 24 months old rats. In addition, Figures 4-26a and 4-27a represent examples of how the morphometric analyses were conducted to determine glomerular volume density, numerical profile density and proximal tubular surface density in the renal cortex of 1 month old and 15 months old rats. Although these are just two small samples, they do illustrate quantitatively the types of changes typically seen in aging rat kidneys.

In all groups, there was a significant increase ($p \leq 0.05$) with age of inflammation, interstitial fibrosis, glomerular and tubular basement membrane thickening, hyperplasia, and tubular dilation/cast formation. Cd exposure did not have a significant effect on any of these parameters, as assessed by ANOVA and Bartholomew's test for crdered means.

As indicated in Table A-56, the 1 month old rats (i.e., at 0 months exposure time) were free of observable tissue changes, except for an extremely mild increase in interstitial fibrosis. By 9 months, all groups had mild to moderate tissue changes (Table A-57). By 15 months, there was a noticeable increase in the percentage of animals with moderate changes and several had severe inflammation, interstitial fibrosis, tubular dilation and hyperplasia. by 24 months, all groups exhibited a wide range of severity for all tissue parameters studied. Thus, even at this advanced age, many rats in each group had only mild changes, while others had severe tissue damage.

Although Cd exposure was determined not to have a statistically significant effect on the degree of renal tissue change, the data are suggestive of certain dose-response relationships. Examination of the group mean scores of tissue pathology (Table A-56) showed that Cd exposure had the most noticeable effect on the amount of hyperplasia. At each sacrifice point tested, there was a dose-response relationship between Cd exposure and the degree of hyperplasia. This was also seen when the percentages of individuals within each group having moderate to severe hyperplasia were compared at each time point (Table A-57).

A Chi-square analysis was performed on the data from Table A-57. Because of the small sample size per cell, it was not possible to detect significant differences between the groups in terms of the percentage of animals having mild, moderate or severe tissue involvement. Another Chi-square analysis was performed in which the animals having either moderate or severe tissue changes were combined into one group and compared to the numbers of animals having only mild changes. Again, this analysis showed no significant differences ($p \leq 0.05$) between any two exposure groups at any time point.

Correlation coefficients were calculated between both age and concentration of renal Cd and the degree of change of each of these tissue

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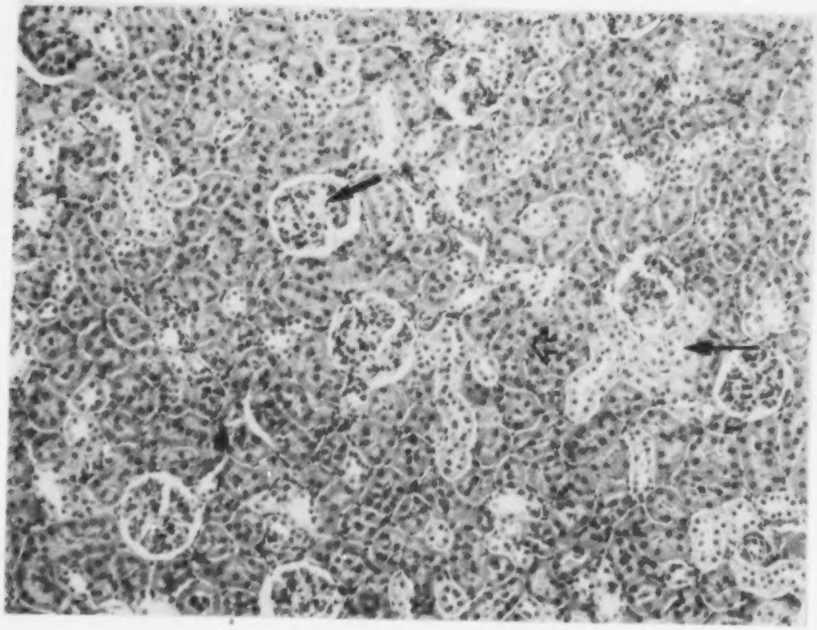


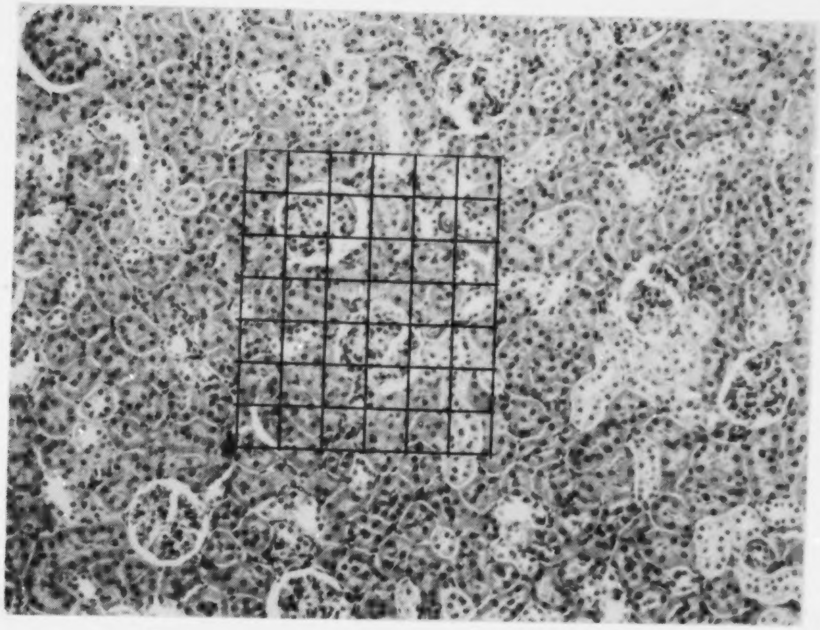
↑ Glomerulus

⇧ Proximal convoluted tubule

↑ Distal convoluted tubule

This rat was sacrificed one week after it arrived and was not exposed to cadmium. The tissue had no measurable pathology except for an extremely mild interstitial fibrosis. (Periodic Acid Schiff; 160 magnification)

Figure 4-26. Kidney Tissue Structure of a One Month Old Rat (Outer Cortex)



(See Materials and Methods section, Figure 3-3 for explanation of methods used for morphometric analysis.)

In this example, the test grid has 56 test points, a total area of 1,672 mm^2, and a horizontal test line 304 mm long.

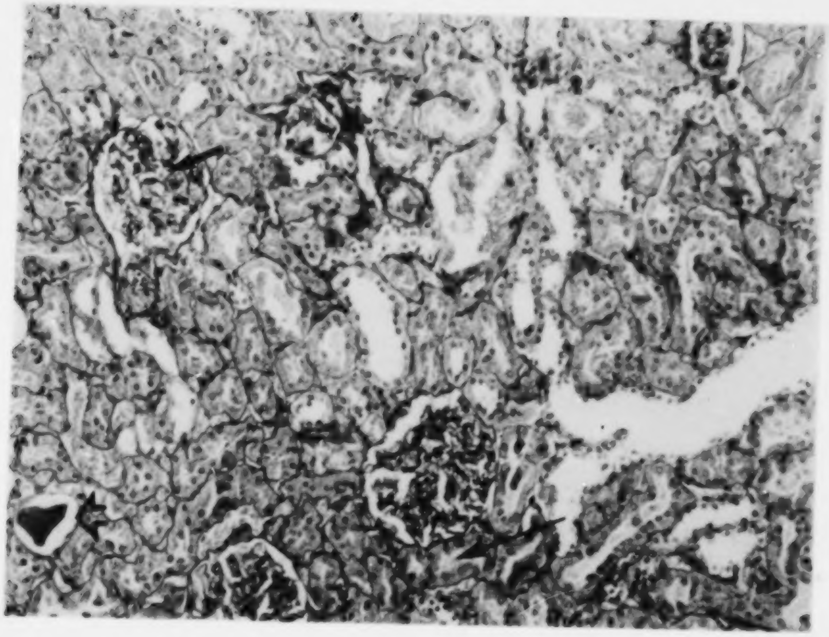
Glomerular Volume Density (Vv) =

$$\frac{\#\ \text{test points within all glomeruli}}{\text{total number of test points}} = 8/56$$

Glomerular Numerical Profile Density (Na) = # glomeruli/grid
= 2/grid
= 2/1,672 mm^2 of cortex

Surface Density of Proximal Tubules (Sv) = (# times tubule wall crosses horizontal test line) ÷ (total length of test line)
= 60/304

Figure 4-26a. Sample Morphometric Analysis of Kidney Structure of a One Month Old Rat (Outer Cortex)



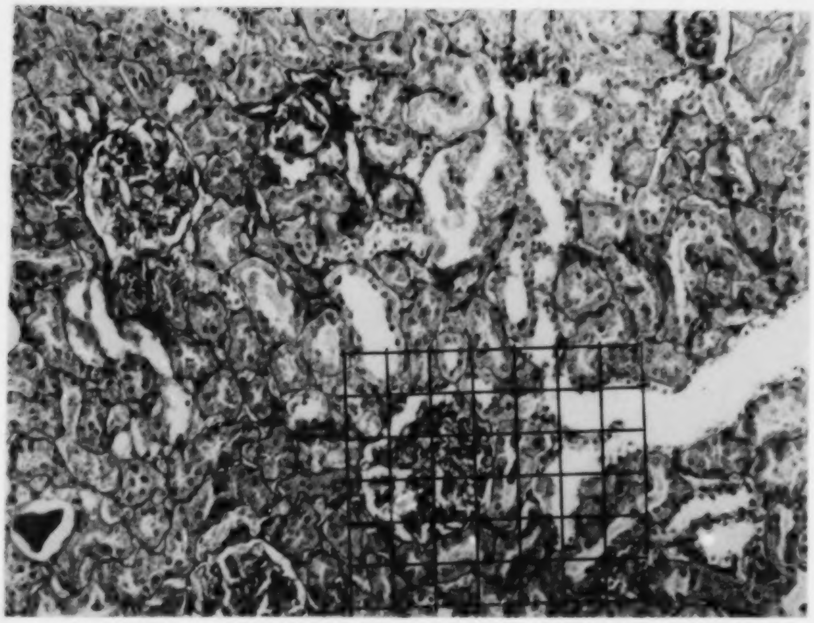
↑ Glomerulus

⇧ Dilated tubule containing cast

↑ Proximal convoluted tubule

This rat was in the Control group. The tissue pathology was graded as follows: inflammation (4), interstitial fibrosis (2-3), glomerular basement membrane thickening (2), tubular basement membrane thickening (2), tubule dilation/casts (2-3), hyperplasia (2-3). (Periodic Acid Schiff; 160 mangification).

Figure 4-27. Kidney Tissue Structure of a Fifteen Month Old Rat (Outer Cortex)



In this example, the dimensions of the test grid are the same as those of the grid in Figure 4-26a.

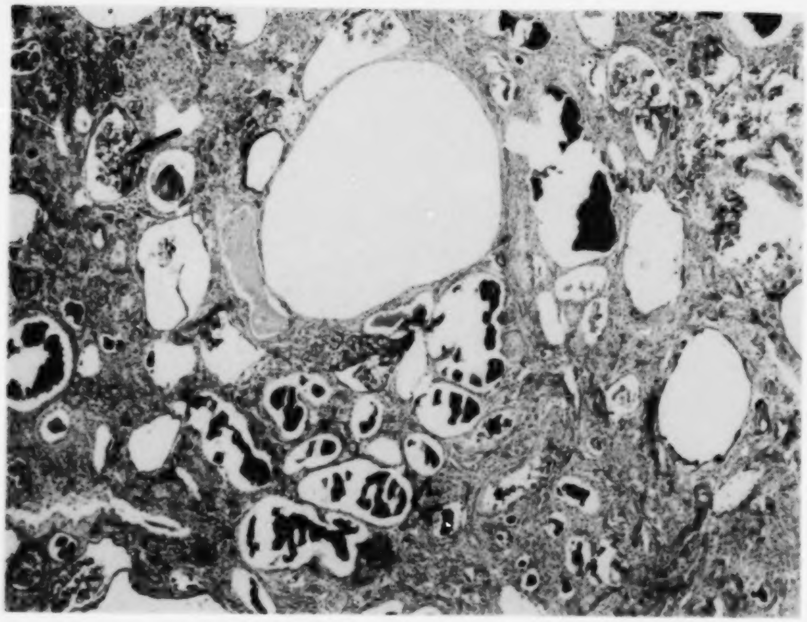
Glomerular Volume Density (Vv) =

$$\frac{\text{\# test points within all glomeruli}}{\text{total number of test points}} = 11/56$$

Glomerular Numerical Profile Density (Na) = # glomeruli/grid
= 1/grid
= 1/1,672 mm^2 of cortex

Surface Density of Proximal Tubules (Sv) = (# times tubule wall crosses horizontal test line) ÷ (total length of test line)
= 48/304

Figure 4-27a. Sample Morphometric Analysis of Kidney Structure of a Fifteen Month Old Rat (Outer Cortex)



↑ Glomerulus

⇧ Dilated tubule containing cast

This rat was in Group 2 (0.5 g Cd^{+2}/ml). The tissue pathology was graded as follows: inflammation (4), interstitial fibrosis (5), glomerular basement membrane thickening (4), tubule dilation/casts (5), hyperplasia (5), tubular basement membrane thickening (4). (Periodic Acid Schiff; 60 magnification)

Figure 4-28. Kidney Tissue Structure of a Twenty-four Month Old Rat (Outer Cortex)

parameters. As expected for each group, there was a significant, strong correlation between age (both 0-15 and 0-24 months) and the severity of tissue damage for each parameter tested. The relationship between renal Cd (μg Cd/gram dry tissue) and the degree of tissue change was not clear-cut, as indicated by the following correlation coefficients:

0 - 24 Months	Control	Group 2	Group 3	Group 4	Overall
Renal Cd:Inflammation	0.34	0.39	-0.12	0.54*	0.32*
Renal Cd:Fibrosis	0.34	0.23	-0.23	0.16	-0.06
Renal Cd:Glomerular Basement Membrane Thickening	0.43*	0.37	-0.13	0.57*	-0.02
Renal Cd:Tubular Membrane Thickening	0.52*	0.61*	-0.07	0.43*	0.05
Renal Cd:Tubular Dilation	0.42*	0.27	-0.25	0.36	0.07
Renal Cd:Hyperplasia	0.27	0.28	0.03	-0.07	0.16

0 - 15 Months	Control	Group 2	Group 3	Group 4	Overall
Renal Cd:Inflammation	0.61	0.67*	0.16	0.63*	0.39*
Renal Cd:Fibrosis	0.48*	0.42	0.14	0.01	-0.08
Renal Cd:Glomerular Basement Membrane Thickening	0.59*	0.72*	0.28	0.58*	0.03
Renal Cd:Tubular Membrane Thickening	0.67*	0.67*	0.30	0.54*	0.04
Renal Cd:Tubular Dilation	0.56*	0.65*	-0.02	0.38	0.06
Renal Cd:Hyperplasia	0.48*	0.77*	0.40	0.02	0.20

*Correlation coefficient significant at $p \leq 0.05$.

See Table 4-3 for calculation of the number rats used to determine these correlation coefficients.

Considering the data as a whole, irrespective of group, there was a significant correlation only between renal Cd levels and the degree of inflammation. Renal Cd levels showed a significant correlation with increased thickening of the proximal tubular membrane for the Controls and Groups 2 and 4, but not for the data as a whole. Dilation and cast formation in the proximal tubules correlated with renal Cd only for the Controls (0-24 months) or the Controls and Group 2 (0-15 months).

Tissue damage indicative of Cd toxicity, such as cloudy swelling or atrophy of proximal tubule cells was not prominent.

OCCURRENCE OF TUMORS

Although the present work was not designed as a carcinogenic study, grossly visible masses from animals that died naturally or were sacrificed as part of the study were removed and examined microscopically. In addition to several subcutaneous fibromas and lipomas, the following skin neoplasms were found:

	Control	Group 2	Group 3	Group 4
Keratoacanthoma	2	1	5	1
Zymbal's Gland[a] Carcinoma	1	1	1	1
Squamous Cell Carcinoma		1	1	1
Sebaceous Adenoma			1	
Basal Cell Carcinoma				1

[a] Zymbal's gland is a sebaceous gland near the base of the rat's ear.

The fibromas and lipomas are both benign neoplasms seen in aging rats.

There was no significant difference ($p \leq 0.05$) in the skin tumor incidence between any of the groups, as determined by a Chi-square analysis.

SECTION 5

DISCUSSION

EFFECTS OF CADMIUM EXPOSURE ON GENERAL HEALTH

The effect of Cd exposure on the general health status of the rats has been assessed by several factors, including body and organ weights, hematocrit, and food and water consumption.

Increased Cd exposure resulted in decreased food and water consumption, body weight and hematocrit. Reduction in the values of all three of these variables would indicate that Cd exposure adversely affected the general health of the individual. However, it was noted in this study, as in others (Berg, 1965), that water consumption patterns also change with age, irrespective of Cd exposure.

An inverse relationship between the levels of Cd in the drinking water and the volume of water consumed appears to be a common finding in animal studies (Perry *et al*., 1977; Cory-Slechta and Weiss, 1980). It has been suggested (Cory-Slechta and Weiss, 1980) that reduced fluid intake, with concomitant reduction in food intake and weight gain, results more from a taste aversion to Cd rather than from an adverse physiological reaction to the metal. If taste aversion is a confounding variable, then a Control group would have to be used that was given quantities of water and food equivalent to those consumed by the dosed rats in order to differentiate the health effects resulting from Cd exposure from those resulting from reduced nutrition.

A further confounding variable is the finding that poor nutrition, as reduced protein intake, can cause anemia in rats (Hardy, 1967). Thus the reduced food consumption in the study rats may have contributed to the reduced hematocrit levels in the high dosed group. However, elevated dietary Cd in animals interferes with the metabolism of the essential metals Zn, Cu and Fe, and as such results in various physical problems, including reduced growth and anemia (Bremner, 1979). In view of these findings, it seems probable that the reduced food and water intake, body weight and hematocrit of the present study resulted from a combination of factors, including physiological responses not only to Cd, but also to a poor nutritional status caused by a dislike for the taste of the dosed water and a consequent reduction of fluid intake.

Interestingly, the total wet weights of the liver, spleen, heart, intestine, lung and kidney were not significantly affected by Cd exposure. Also, with the exception of the kidney, the weights of these organs, expressed as the percentage of the body weight, were not significantly altered by Cd exposure. Moreover, with the exception of the spleens of Group 4 animals, none of the other organs had a measurable increase in tissue fluid as a result of Cd exposure. In general, the data seem to indicate that the health status of these rats probably was not severely compromised at the levels of Cd tested.

RENAL METAL KINETICS

Tubular Versus Cortical Metal Concentrations

Data from this study support the hypothesis that the renal cortical tubules preferentially accumulate Cd, Zn and Cu. The cortical tubule preparations were highly enriched for proximal tubule segments and, although not pure, they essentially were free of glomeruli, fibrous tissue. These data also are consistent with the findings of previous studies using chronically exposed animals (Kubota et al., 1974; Kyohno and Kawai, 1975; Nishizumi, 1975; Murakami et al., 1975) in which x-ray microanalysis and various staining procedures of thin kidney sections showed Cd to localize in the cortical proximal tubular epithelium. Also, since the proximal tubules are rich in enzymes and form a site of high metabolic activity and extensive material transport, they would be expected to be rich in essential metals such as Cu and Zn.

The kinetics of metal storage determined not only for the kidney but also for the liver, intestine, heart and spleen are consistent with the findings of Stonard and Webb (1976), Danielson et al. (1982, 1982a) and others indicating that metallothionein is involved in the tissue accumulation of Cd. Metallothionein, whose production is induced by Cd, binds to Zn and Cu, resulting in the tissue accumulation of these two metals.

The present study demonstrated that increased renal Cd correlated with increased renal Zn and Cu. However, Cd had a far more significant effect on the levels of renal Cu than renal Zn. Also, increased hepatic Cd resulted in significantly higher levels of hepatic Zn but had no effect on hepatic Cu. Further, increased concentrations of Cd in the intestine, heart and spleen had no significant dose-response effect on the levels of Cu or Zn in any of these organs. Stonard and Webb (1976) found very similar results in their Cd feeding study with Wistar rats. Additionally, they isolated high levels of Cu-thionein from the kidney and Zn-thionein from the liver of dosed animals and concluded that the increased levels of these two metals correlated with increases in the liver and renal metallothioneins. In contrast, these authors were unable to isolate Cu-thionein or Zn-thionein from the intestine even though this organ accumulated Cd.

Danielson et al. (1982), using staining techniques, showed that in the small intestine, metallothionein localized only in the surface of columnar epithelial cells of the villi, with the exception of the goblet cells. This was not a quantitative procedure for metallothionein and the authors did not isolate specific Cu- or Zn- binding protein. Stonard and Webb (1976) also noted that other workers (Webb and Verschoyl, 1976) found low concentrations of Cd in the spleen and hearts of animals continually exposed to ingested Cd; however, the concentrations of Cu and Zn were not disturbed and measurable amounts of metallothionein were not detected. In view of these findings, the conclusions of Stonard and Webb (1976) seem applicable to the present study. These authors suggested that the local accumulation of Zn in the liver and Cu in the kidney was due to the Cd-induced synthesis of thionein and did not cause systemic deficiency of either of these essential metals. This view that systemic deficiency of Cu and Zn is not induced is also consistent with the present study. At the levels tested, it was found that although Cd increased significantly in the lungs of the high dosed rats (approximately 2.0 μg/g dry weight), there was no alteration in the Cu or Zn concentrations in this organ. Also, trace amounts of Cd were found in the muscle of the high dosed rats but Cu and Zn were not affected.

The present study also showed that, although Cd exposure disturbed the normal distribution of Cu and Zn in the kidney and Zn in the liver, there appeared to be a threshold of Cd concentration necessary to cause this response. The levels of renal Cd in the 0.5 and 5.0 μg/ml dosage groups were insufficient to alter significantly the levels of cortical Cu or tubular Cu and Zn. On the other hand, the levels of Cd in the 50 μg/ml group were high enough to cause a significant increase in the concentrations of Cu and Zn in the kidney and Zn in the liver. These findings also are consistent with the role of an inducible metalloprotein mediating the accumulation of tissue metals.

The kidney and liver both have the capacity to store large amounts of Cd and together they form the major reservoir of the body burden of this metal. The present study indicated that the partitioning of Cd between these two organs may be dose dependent. At the levels tested, the concentration of Cd (μg/g dry weight) in the kidney was consistently higher than that in the liver for all dosage groups. This is similar to the findings of others (Perry et al., 1977; Kjellstrom and Nordberg, 1978; Gross et al., 1976) who determined that renal Cd concentrations exceeded hepatic Cd concentrations in animals exposed to low doses of Cd and in humans exposed to Cd in the environment. In terms of total organ Cd burden (as μg Cd/organ), the present study indicates that the higher the exposure dose, the higher the total amount of Cd sequestered in the liver as compared to the kidney. Thus, the liver organ burden exceeded the renal organ burden from 0-6 months in Group 2, from 0-9 and 21-24 months in Group 3, and for the entire study in Group 4. This suggests that the higher the total body burden, the higher the percentage that is stored in the liver as opposed to the kidney. These findings are consistent with the estimate suggested by Friberg et al. (1974). These authors suggested that for people not occupationally exposed to inhaled Cd, approximately 50% of the Cd body burden is located in the liver and kidneys together and 33% is in the kidneys alone, which implies that the renal organ burden exceeds the hepatic organ burden. However, they also noted that for workers exposed to Cd, and animals exposed to high levels of Cd, a larger proportion of the body burden will be found in the liver as compared to the kidney.

The present study also suggested two interesting relationships between the rates of accumulation of metal in the kidney and the magnitude of the dose. First, the linear portion of the curves for the concentration of Cd in the renal cortex were used to calculate the group rates of accumulation of the metal in the kidney. It was found that the rate of Cd accumulation in the cortex increased by a factor of 5 between Groups 2 and 3 as compared to a factor of 8 in the actual dose to the rats. Also, the cortical Cd accumulation rate increased by a factor of 8.6 between Groups 3 and 4 as compared to an increase in the actual dose to the rats of 7.8. These observations indicate that the rate of accumulation of Cd in the cortex did not increase linearly with the actual dose to the rats. These results suggest that for the exposures tested, the actual dose to the rat cannot be used to predict the cortical concentrations of Cd at a given time.

Second, the relative rate of accumulation of Cd in the cortical tubules versus the intact cortex was calculated for each group for the linear portion of the Cd accumulation curve (i.e., 0-12 months for Groups 2 and 3, and 0-15 months for Group 4). The ratios of the rates of cortical tubule:cortical accumulation were found to be 1.2, 1.3 and 1.4 for Groups 4, 3 and 2, respectively. Although the differences in these ratios are very small, they do show a dose-related trend. This would suggest that the rate of accumulation of Cd in the intact cortex may not be a good indicator of the rate of accumulation of the metal in the cortical tubules at all doses. It does suggest, however, that at higher doses, the rate of Cd accumulation in the intact cortex would be a good estimator of the rate of accumulation in the cortical tubules. Since the preparation of tubule pellets is extremely time-consuming, it seems reasonable to use the cortical data as an approximation for the cortical tubule data.

Effects of Age on Metals Accumulation in the Kidney

The profiles of renal Cd concentration versus time have shown that in all exposure groups, cortical and cortical tubular Cd concentrations increase with time of exposure up to 15 months and then begin to decrease. The 15 month time point appears to be a critical time for Cd kinetics independent of the renal Cd burden, thus suggesting that the effect is related more to aging than to Cd toxicity. In a similar manner, other chronic Cd feeding studies in animals (Perry *et al*., 1977; Nomiyama, 1974; Kajikawai *et al*., 1981) have demonstrated that renal Cd concentration correlates positively with age and increasing doses in young and adult animals but then decreases in old individuals. The same effect was seen in renal autopsy tissue from people not exposed occupationally to Cd (Travis and Haddock, 1980; Gross *et al*., 1976; Piscator and Lind, 1972).

The present work also indicated a similar age factor in the renal accumulation of cortical tubular Zn, and tubular and cortical Cu. Comparison with the Control rats showed that Cd exposure elevated renal Cu and Zn in a dose dependent manner. As with Cd accumulation, tubular and cortical Cu and tubular Zn levels peaked around 15 months and declined thereafter.

It was found that the patterns of metal accumulation in the whole cortex differed somewhat from those in the tubules, particularly for Zn. This may be due to the fact that the intact cortex contained a certain amount of non-tubular tissue, such as glomeruli, blood vessels, fibrous tissue, etc., that also sequester these metals to varying degrees. On the other hand, the cortical tubule preparations were largely free from these non-tubular tissues and their metals. Further, as shown by the morphometric analysis, the proportion of tubular and non-tubular tissues in the kidney cortex changes as a function of age. Therefore, it is not unreasonable that the profiles of metal accumulation in the cortical tubules should be somewhat different from those in the whole cortex.

By separately analyzing the cortical tubules and the whole cortex, it was hoped that the cause of decreased cortical Cd levels with old age could be explained. It was theorized that if the cortical levels declined with age but the cortical tubular levels plateaued or continued to increase, then the decreased cortical levels could be attributed to the age-related infiltration of renal fibrous tissue, which concentrates minimal amounts of Cd. The data partially support this theory. First, the morphometrics showed that there was a significant decrease with age of the numerical density of glomeruli and, therefore, the number of nephrons. Second, there was a significant age-associated decrease in the volume density of intact proximal tubular walls in the outer cortex. Third, there was a significant age-associated increase in the amount of interstitial fibrotic tissue. All three effects were seen equally among the Controls and the dosed animals.

These data suggest that the decrease of renal Cd with age is due to at least two factors. First, with age, there is an actual physical loss of intact proximal tubules, the component of the renal cortex that sequesters the highest proportion of the Cd organ burden. Second, with increasing age, there is a change in the proportion of tissue types in the kidney. Presumably, each tissue type has a characteristic metal burden so that alterations in the tissue composition could lead to alterations in the renal metal levels. Given the present data, these factors seem to apply to the accumulation patterns of Zn and Cu as well as Cd.

It was found that, after 15 months, the concentrations of all three metals in the cortical tubules decreased significantly. This may be the result of several factors. One plausible explanation is that tubules or tubular cells having the highest Cd concentrations were constantly being lost from the kidney, leaving behind intact tubules with lower Cd, and hence lower Zn and Cu concentrations. In support of this view, Kajikawa et al. (1981) have shown that, in rats displaying renal morphological changes associated with Cd toxicity, all parts of a single tubule or even a single tubular cell were not affected to the same degree. They also found that individual tubules and tubular cells within a single kidney also reacted to Cd to various extents.

The cortical tubular Cu levels of Group 4, which at their peak were three times higher than the Control levels, showed a precipitous decline after 15 months. By 24 months, Group 4 cortical tubular Cu concentrations were not significantly higher than Control levels. In contrast, Group 4 cortical tubular Cd levels also declined after 15 months; however, by 24 months, they remained significantly elevated above Control values. These observations suggest that whatever the mechanism of Cd toxicity, the most susceptible cells may be those having elevated Cu concentrations. Interestingly, Suzuki (1981) found that in Cd dosed rats, during the period of latent toxicity, Cu-metallothionein appeared in the urine before measurable quantities of Cd were detected. Further, measurable Cd did not appear in the urine until there was Cd-induced renal damage.

The present findings correlate with the theory of Travis and Haddock (1980) that, in man, not occupationally exposed to Cd, age-related changes in the structure and function of the kidney have the greatest affect on the increased elimination of urine Cd and the decreased concentration of renal Cd.

RENAL FUNCTION

In general, the data indicate that Cd, at the doses tested, had little adverse effect on the functioning of the kidney. Significant dose-response relationships were not anticipated since the maximum cortical concentrations of Cd were approximately 90 µg/g wet weight (in Group 4) and it has been suggested (Friberg et al., 1974) that 200 µg/g wet weight is the critical concentration associated with detectable renal dysfunction. Further, as pointed out by Kluwe (1981), in a nonstressed state, the mammalian kidney utilizes only a portion (about 20-25%) of the total renal capacity. He suggested that, because of the kidney's large functional reserve, standard renal function tests may be insufficiently sensitive to detect subtle toxic effects.

Age alone caused more significant changes in renal function than did Cd exposure. Interestingly, for certain parameters, including diurnal urine volume, urine glucose and specific gravity, the effect of old age in combination with high Cd exposure appeared to have a greater effect on renal function than did age alone. Thus, the data were suggestive of a mild toxic effect of Cd expressed only in late life.

The rat presented a picture of gradual renal change with time that included decreased levels of renal and urine LAP, proteinuria, changing diurnal urine volumes, decreasing pH, and decreasing specific gravity.

Unlike the findings of Cousins et al. (1973) and Vigliani (1967), the present work showed no correlation between Cd exposure and reduced activity of renal LAP. In contrast, the data indicated that the renal enzyme activity was related to age, being higher in young animals and then decreasing with age. Cousins et al. (1973) demonstrated a significant reduction in enzyme activity in porcine kidneys; however, the concentration of renal Cd was greater than 200 µg/g wet weight of cortex and the measurements were made at only one time point so that age-related trends were not derived.

The present work also demonstrated a decrease in urine LAP excretion that correlated with decreased levels of renal LAP. This observation suggests that older rats synthesize less enzyme. In view of previous studies (Ono et al., 1968; Goldbarg and Rutenberg, 1958), it was anticipated that older rats having nephritis, and Cd-dosed rats having tubular dysfunction would have reduced renal LAP but increased urine LAP. Such was not the case. However, among the individuals within a group, a large variance was found in the levels of urine LAP. That some individuals had very high levels, while others had relatively low levels may have been a reflection of the varying degrees of renal pathology.

As expected, proteinuria showed a significant association with age (Berg, 1965; Anver and Cohen, 1979). At the levels of Cd tested, there was no significant dose-response relationship between proteinuria and exposure. However, throughout most of the experiment, the mean daily protein excretion in Group 4 exceeded that in the Controls, although the difference was not statistically significant. As noted in the Results section, the combination of small sample size and large variance made it difficult to detect significant dose-response relationships of some parameters. Thus, the data may have indicated a trend towards a mild renal dysfunction with increased Cd exposure.

Changes in the diurnal urine volume and specific gravity also may indicate a mild renal dysfunction found only in the old, high dosed rats. In the Control and low dose rats, there was an age-related trend towards increased urine volume during the first 10-15 weeks, followed by a period of decreased volumes. Generally, urine volumes increased again in the old rats. The situation in the high dosed rats was significantly different. For the experiment as a whole, Group 4 rats voided significantly smaller urine volumes compared to the Controls and low dosed groups, presumably as a result of their reduced water intake. Because these rats were able to excrete small volumes of highly concentrated urine indicates that their renal concentrating ability, which is a tubular function, was not significantly impaired by Cd exposure (Koushanpour, 1976; Kurtzman and Rogers, 1974).

After about week 50, there was a noticeable, steady rise in urine volume and a decrease in specific gravity for the Group 4 rats. Interestingly, the correlation coefficients relating age to urine volume for 0-15 months indicated a significant ($p \leq 0.05$) negative correlation for each group. However, when these coefficients were recalculated for 0-24 months, to include the old rats, only Group 4 showed a significant correlation, and it was positive ($r = 0.26$, $p \leq 0.05$).

All groups showed a similar age-related trend for urine specific gravity. This parameter increased in value up to week 50-70, with the majority peaking at week 62, and declined thereafter. It is interesting that this change occurred around week 62, which is equivalent to 15.5 months, the point at which the renal metal concentrations peaked.

In a situation similar to that for the urine volume, when the old rats were included, only Group 4 showed a small, but significant correlation ($r = -0.23$, $p = 0.072$) between age and decreasing urine specific gravity. These observations suggest that higher Cd dosing in combination with old age may have a mild adverse effect on tubular ability to concentrate urine. Significant correlations have been found between impaired urine concentrating ability and both high dietary and occupational Cd exposures in humans (Becker *et al.*, 1971; Kazantzis, 1979b; Chang *et al.*, 1981).

Finally, increased urine excretion of glucose after week 50 in Groups 3 and 4 may be indicative of a mild Cd-related renal tubular dysfunction. There was no significant difference in daily glucose excretion between any of the groups; however, there was a significant correlation between age and urine glucose for Groups 3 ($r = 0.30$, $p \leq 0.05$) and Group 4 ($r = 0.46$, $p \leq 0.05$) for 0-24 months. There were no significant correlations between age and urine glucose for the Controls or Group 2 at 0-24 months or for any of the groups at 0-15 months. These observations imply that age, in combination with higher Cd dosing resulted in mild impairment of the proximal tubular reabsorption of glucose. Others (Axelsson and Piscator, 1966; Yoshikawa, 1967) have found glycosuria to appear concurrently with or shortly after the onset of proteinuria in Cd-exposed animals. In view of this, it was interesting to note that around week 50, there was a noticeable increase in the excretion of urine total protein in all groups and urine glucose in Groups 3 and 4.

RENAL TISSUE STRUCTURE

As expected, there were many age-related changes in the kidney tissue structure of all study groups. Consistent with the findings of others (Snell, 1967; Coleman, et al., 1977; Lowenstein, 1978; Arataki, 1926; Anver and Cohen, 1979), age correlated significantly ($p \leq 0.05$) with increased inflammation, interstitial fibrosis, glomerular and tubular basement membrane thickening, tubular dilation/cast formation, and hyperplasia. Further, the glomeruli became significantly ($p \leq 0.05$) larger with age, but their density in the outer cortex became significantly smaller. Although there were no statistically significant differences between the groups, Group 4 rats, in comparison to Controls, tended to have a higher percentage of individuals with moderate to severe hyperplasia and tubular dilation/cast formation.

The present data suggest that Cd exposure may have had a toxic effect on the kidney that was expressed by an increase in renal mass. Although there was no statistically significant difference between the groups in the total wet weight of the kidneys, Group 4 consistently had heavier kidneys than all other groups throughout most of the experiment. Also, when the kidney weight was recalculated as the percent of body weight, Group 4 kidney values were significantly higher ($p \leq 0.05$) than those of the Controls. That the renal wet/dry weight ratios were not significantly different between the groups indicated that increased tissue fluid did not cause the increased kidney weight in Group 4.

As noted previously, Group 4 rats weighed significantly ($p \leq 0.05$) less than the Controls; therefore, it was expected that their kidneys would be proportionately smaller. However, the exact opposite was found. Interestingly, of all the organs examined (i.e., liver, intestine, spleen, heart, lung, kidney), only the kidneys of Group 4 and the Controls were significantly different in terms of their mass relative to body weight.

Since increased fluid content was ruled out, increased hyperplasia in Group 4 seemed the likely cause of the larger kidneys. Morphometric analysis supported this idea. Although the differences were not significant, Group 4 consistently scored higher than Controls when the degree of hyperplasia was ranked (0-5 scores) and averaged for the groups. Further, although not statistically significant, the percentage of rats having moderate to severe hyperplasia was consistently higher in Group 4 compared to the Controls for each time point examined. For example, by 24 months, 75 percent of Group 4 and 50 percent of Controls had moderate to severe hyperplasia.

Age also had an apparent effect on the structural integrity of the renal cortical tubules in all groups. Prior to 21 months, the tubule preparations used for metals analysis routinely consisted of short tubule segments with few glomeruli and little cell debris. From 21 to 24 months, most of the preparations were of a poor quality, containing larger proportions of cell debris and glomeruli and smaller amounts of tubule segments. Some preparations had few, if any, tubule segments. As this change was seen in all the study groups, it is probable that the tubules had grown more fragile with age and were unable to withstand the enzyme digestion and centrifugation procedures.

Although the total number of glomeruli per kidney could not be calculated, the glomerular size, volume density, numerical profile density and numerical density were estimated. The values of these parameters showed trends that were consistent with the findings of Arataki (1926). He determined the total number, size, volume density and numerical density of glomeruli in the kidneys of normal male and female Wistar rats from newborn to 500 days of age. The average diameters of glomeruli in the present study compared very favorably to those determined by Arataki (1926). Average diameters for the present study versus Arataki's study were as follows: 75 versus 68 μm (1 month); 113 versus 111 μm (9 months) and 126 versus 124 μm (15 months).

Although Cd exposure did not have a significant effect on the mean size of the glomeruli, it had a mild, but noticeable effect on the size distribution of these structures. Thus, in comparison to Controls, a greater percentage of the rats in Group 4 had larger glomeruli. Because of the small sample size (N = 24 rats per group), statistically significant differences could not be detected.

Because of differences in measurement techniques, the absolute values of glomerular volume density and numerical density could not be compared to those calculated by Arataki (1926).

The glomerular volume density was not significantly different among the present study groups, nor was it affected significantly by age. These findings are in agreement with those of Arataki (1926) for his rats aged 30-500 days. Also, the numerical density of glomeruli in the present study was significantly ($p \leq 0.05$) reduced with age, but not significantly affected by Cd dosing. This observation was in agreement with Arataki (1926), who found that from 30 to 500 days of age, the normal male rat glomerular density (number glomeruli/mm^3 cortex) declined by 75 percent.

Since the trends in the present study parallel those found by Arataki (1926), it seems reasonable to assume that, as in his study, the total number of glomeruli per kidney decreased with age. Even though the glomerular volume density, numerical profile density and numerical density were not significantly affected by Cd exposure, it is not possible to postulate, given the existing data, whether Cd exposure altered the total number of glomeruli per kidney.

At the levels tested, renal morphological changes typical of Cd toxicity were not detected. This was not surprising since reports in the literature of Cd-induced tissue changes vary considerably for both high and low exposures. Further, as noted by both Kawai *et al*. (1976) and Kajikawa *et al*. (1981), mild Cd-related kidney damage may easily be obscured by extensive tissue pathology due to aging.

SECTION 6

CONCLUSIONS

Data from this study have suggested many interesting age-related renal changes which alter the kidney's physiological handling of the toxic metal, Cd, and the essential metals, Cu and Zn.

The hypothesis that chronic Cd exposure influences the physiological, morphological, and biochemical aging of the kidney has been supported partially by this study. A clear-cut proof of this hypothesis was not fully supported because of the levels of Cd exposure tested and because of the statistical problems with the small sample size, the obtained large variances, and the supply of two dissimilar populations (i.e. early vs. late rats). In spite of these problems, many interesting trends were shown by the data. In general, toxic effects from Cd exposure were mild and limited to Group 4, the highest dose group.

The health status of the high dosed rats, as measured by hematocrit, body weight gain, and food and water intake, was compromised. Physiological responses to both Cd and a poor nutritional status caused by a dislike for the taste of the treated water were a likely cause of this condition.

The data have supported the hypothesis that the renal cortical tubules preferentially accumulate Cd, Zn and Cu relative to the whole cortex. In general, the cortical tubular metal concentrations were significantly higher than cortical concentrations at least through 18 months of exposure. Thereafter, tubular concentrations decreased and became equivalent to or significantly less than cortical levels. Two mechanisms were proposed to explain this change. First, susceptible tubular cells containing high concentrations of Cd and possibly Cu were lost from the tubule populations, leaving behind cells with lower, less toxic concentrations of metals. Second, cortical tubules from kidneys of old rats were more fragile, as evidenced by the poor quality of the tubule preparations. Metal could have been lost from the disintegrating tubules during the pellet preparation.

Cd exposure disturbs the normal distribution of Cu and Zn in the kidney. The concentrations of these two essential metals are increased in both the cortical tubules and the intact cortex in a dose-response manner, with a much greater effect on the Cu levels. The data support the findings of others who have shown that metallothionein is involved in the accumulation of renal and hepatic Cd, plus renal Cu and hepatic Zn.

The accumulation patterns of Cd, Zn and Cu in the renal cortical tubules appear to differ from those in the intact cortex. Results of the morphometric analysis suggest that these differences may be caused by age-dependent variations in the amount of non-tubular cortical tissue. This non-tubular component consists of interstitial connective tissue that sequesters far less Cd than do the cortical tubules.

The relative rate of Cd accumulation in the cortical tubules versus the intact cortex (as measured by the ratio of the slopes of the accumulation curves up to 12 or 15 months) was dose dependent. Therefore, the cortical Cd concentration cannot be used to predict cortical tubular Cd concentrations at all doses.

There appeared to be definite age-related changes in the kidney which altered this organ's handling of Cd, Zn and Cu. The major changes appeared in the very young (i.e. up to 3 months) and adult rat (i.e. 12-15 months). In the young rat, there was a rapid increase in the concentration of metals which corresponded to the time of most rapid organ growth. In the adult, there was a peak, then a noticeable decrease of renal metal concentrations. This sudden decrease appeared to be linked to an ongoing aging process which slowly abliterated healthy nephrons and changed normal tubules into scar tissues and dilated, cast filled conduits. The same effect is seen in human autopsy tissue from people not exposed occupationally to cadmium (Travis and Haddock, 1980). Thus, in the human kidney, cadmium increases steadily from birth, peaks around age 50 years, and decreases thereafter.

Morphometric analysis has shown that, with age, there was a significant decrease in the amount of healthy proximal tubular tissue, and the number of glomeruli (as measured by the glomerular numerical density). Coincidentally, there were significant increases in the degree of tissue inflammation, the amount of interstitial fibrosis and hyperplasia, the extent of tubule dilation/cast formation and the thickening of glomerular and proximal tubular basement membranes. Cd exposure at the levels tested showed no significant dose-response effects with the occurrence of any of these features. There was, however, a greater prevalence of moderate to severe hyperplasia and tubular dilation in Group 4 as compared to the Controls. There appeared to be a small, but definite shift toward larger glomeruli with high Cd exposure.

Also, there was a significant ($p \leq 0.05$) correlation between renal cortical Cd levels and inflammation of the renal tissue. However, this relationship is confounded by the finding that both inflammation and renal Cd concentrations also correlate positively with age.

In order to determine whether or not Cd exposure influenced the aging process of the kidney, each variable was examined for age effects. Those variables having an age-related component then were examined to determine if they also exhibited a dose- response relationship with Cd exposure. Of all the parameters studied, age and Cd exposure had the most significant influence on the renal handling of Cd, Cu and, to a lesser extent, Zn.

At the levels tested, Cd exposure had a mild effect on renal aging in terms of disturbing the tubular reabsorption of proteins, and altering both the physical integrity of the renal cortical tubules and the size of the glomeruli. Age alone did not appear to have a significant effect on diurnal urine volumes or excretion of urine glucose. However, old age, in combination with high Cd exposure, showed a significant, positive correlation with increased urine volumes and increased glucose excretion. These observations suggest that Cd exposure in the older animals compromised renal tubular functioning to a certain extent.

Cd exposure did not appear to significantly affect age-associated changes in the kidney structure such as increased inflammation, glomerular and tubular basement membrane thickening or interstitial fibrosis. On the other hand, Cd exposure did appear to have a mild effect on renal structure as evidenced by a dose-response relationship with the degree of tubular hyperplasia.

Fifteen months of exposure appeared to be the critical time point for renal metal kinetics as evidenced by the peak in the metals accumulation curves. Interestingly, it also may have been a point at which the renal physiology was changing in other ways, as evidenced by changes in certain renal function tests. Around this time, there was a noticeable increase in the rate of urine total protein excretion. Diurnal urine volumes, which previously had been decreasing, started to increase again. Urine specific gravity peaked at this point and then started to decline again. In Groups 3 and 4, excretion of urine glucose started to increase at this point, and continued increasing through the end of the study.

In examining the relationship between the level of Cd exposure and the concentration of Cd in the kidney, it was found that the rate of accumulation of Cd in the whole cortex did not increase linearly with the actual dose to the rats. These results implied that the actual dose to the rat could not be used to predict the renal Cd concentration at a given time.

The results of this study indicate the need for further research in order to more fully answer the question of whether or not Cd exposure influences normal renal aging. Moreover, a critique of this study points to several factors which must be considered in any future research.

The present data have demonstrated that renal concentrations of 90 μg Cd/g wet weight of cortex are associated with mild functional and structural changes in the kidney in association with old age. Since these data are suggestive of a possible effect of Cd on renal aging, further research is needed using higher exposures. Exposures which would result in attaining the critical concentration of 200 μg Cd/g wet weight in the cortex should be used in conjunction with morphometric techniques for analyzing renal tissue pathology. Also, appropriate measurements of the total amount of cortical tissue in the fresh and fixed kidney should be made so that an estimate of the total number of nephrons and total mass of proximal tubular tissue per kidney can be calculated. Estimates of the total amount of a specific tissue type rather than the proportion of that tissue type in the kidney may result in a more sensitive measure of the effects of Cd exposure on renal structure and aging.

In view of the problems encountered with the statistical analysis of data in this study, it is suggested that future studies use larger numbers of animals at each data collection point. Also, all of the study animals should be received at one point in time. Problems with large interindividual variation between animals may be reduced by using an inbred strain of animals.

The data indicated that reduced water and food consumption in the high dosed rats may have been a confounding variable that adversely affected the general health of the animals. In order to control for this, future work should include the use of a matched Control group which is given food and water in portions equivalent to those ingested by the exposed animals.

The data have shown that the greatest combined effect of Cd exposure and aging concerns the accumulation of Cd, Zn and Cu in the renal cortex. Since 15 months of exposure appeared to be the critical time point for renal metal kinetics in these animals, it would be of interest to examine the metals accumulation in the cortical tubules and intact cortex around this time point but at intervals shorter than 3 months. Moreover, the procedure presently used to separate out the cortical tubules is very time-consuming and results in quite low yields of tubular tissue. Therefore, it is suggested that alternative methods be tested for assessing the concentration of metals in the cortical tubules versus the intact cortex. A potential approach would be to stain fixed kidney sections for Cd, Zn and Cu and then use morphometrics to determine the partitioning of each metal between the tubular and non-tubular tissue.

Finally, the types of clinical tests used to assess renal functioning should be reviewed. Tests more appropriate to detecting proximal tubular dysfunction should be employed.

SECTION 7

PULMONARY FUNCTION STUDIES ON CADMIUM FED RATS*

Static elastic properties and dynamic air flow properties of lungs from control and cadmium fed rats were measured and morphometric measurements of alveolar size were made in this part of the study. The original hypothesis, based on a report from Petering et al. (1979), was that ingested cadmium might lead to pulmonary emphysema similar to that resulting from inhaled cadmium (Snider et al., 1973). Findings from this study, however, did not provide any evidence in support of this hypothesis. Essentially no changes were found in any of the pulmonary function variables measured. Data from each of these tests are presented in Figures 7-1 through 7-7.

In the figures, individual data points for each animal are plotted as functions of cadmium dose and treatment duration. There were four animals in each group. The abscissa has the Control and the treatment groups (0.5 mg/l, 5.0 mg/l, and 50 mg/l cadmium); within each group are the numbers 12 through 24 which refer to the number of months of treatment. Figure 7-1 gives the body weights of the animals in grams. Figure 7-2 gives the wet lung weight in grams. Figure 7-3 provides the total lung capacity (defined as the air lung volume at a transpulmonary pressure of 30 cm H_2O) expressed per 100 grams body weight. Figure 7-4 gives the Percent V_{10} which is defined as the percent volume remaining in the lung at 10 cm H_2O following deflation from 30 cm H_2O. This provides a measurement of the shape of the lung, which can be used as an assay for lung surfactant (Johnson et al., 1964). Figure 7-5 shows the forced vital capacity (FVC) expressed per gram lung weight. The FVC is measured by rapidly applying suction to the trachea following inflation to total lung capacity. Figure 7-6 gives the $FEV_{0.15}$. This variable is measured from the forced vital capacity maneuver and represents the fraction of the FVC which leaves the lung in 0.15 seconds. It is a measure of the small airways resistance in the lung. Figure 7-7 shows the total number of intersections with alveolar walls of a randomly placed grid of parallel lines. The total length of all lines in the grid was 2.072 mm. The number of intersections is inversely proportional to the distance across an alveolus, and hence alveolar size; thus, the larger the number of intersections the smaller the alveoli. The mean number of intersections of about 40 results in a mean alveolar diameter of about 50 micrometers. Tables giving the summary of individual data plotted in the figures are included in Appendix B.

As demonstrated by the data, no effect of ingested cadmium on pulmonary function was found. At present, no satisfactory explanation can be given why Petering et al. (1979) found changes consistent with emphysema. However, it may be said that since they did not do quantitative morphometry, their qualitative judgment of emphysema may not have withstood a more rigorous quantitative analysis.

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*This section was prepared by Wayne A. Mitzner and John M. Frazier, Associate Professors of Environmental Health Sciences, The Johns Hopkins University.

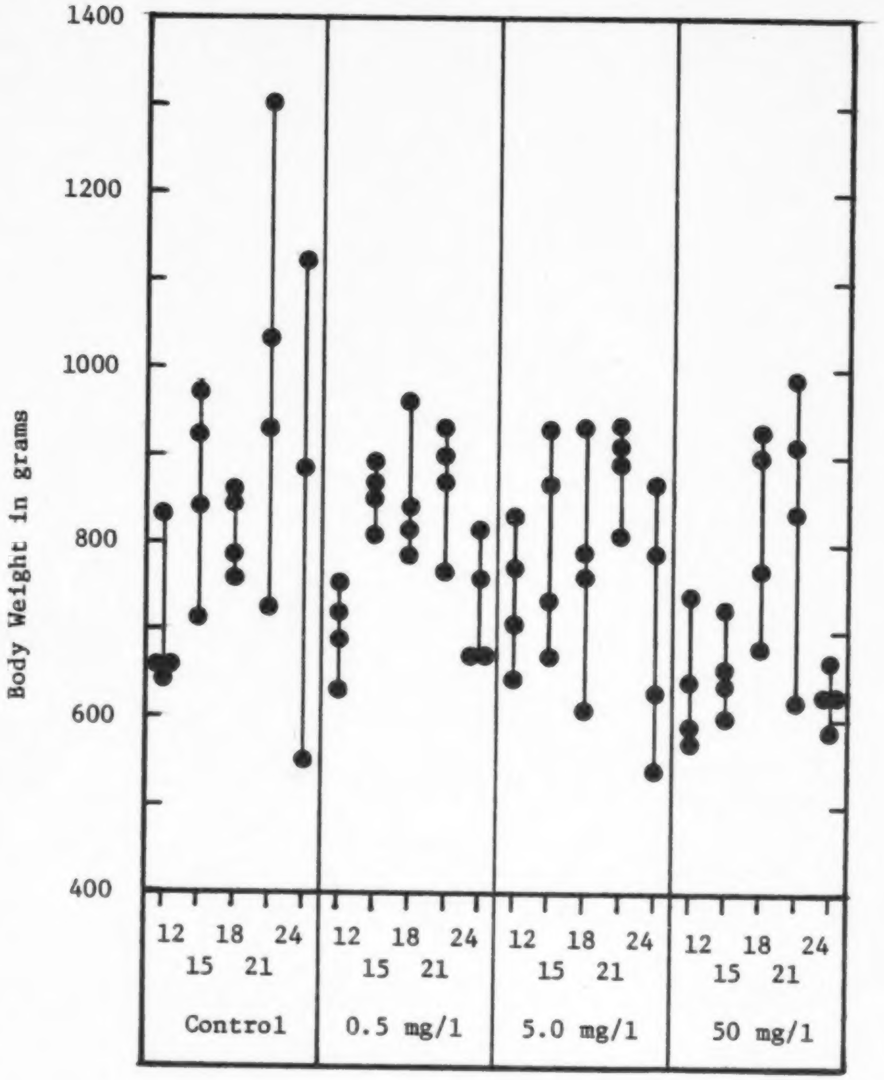

Figure 7-1. Body weight of rats in grams as function of cadium dose and treatment duration.

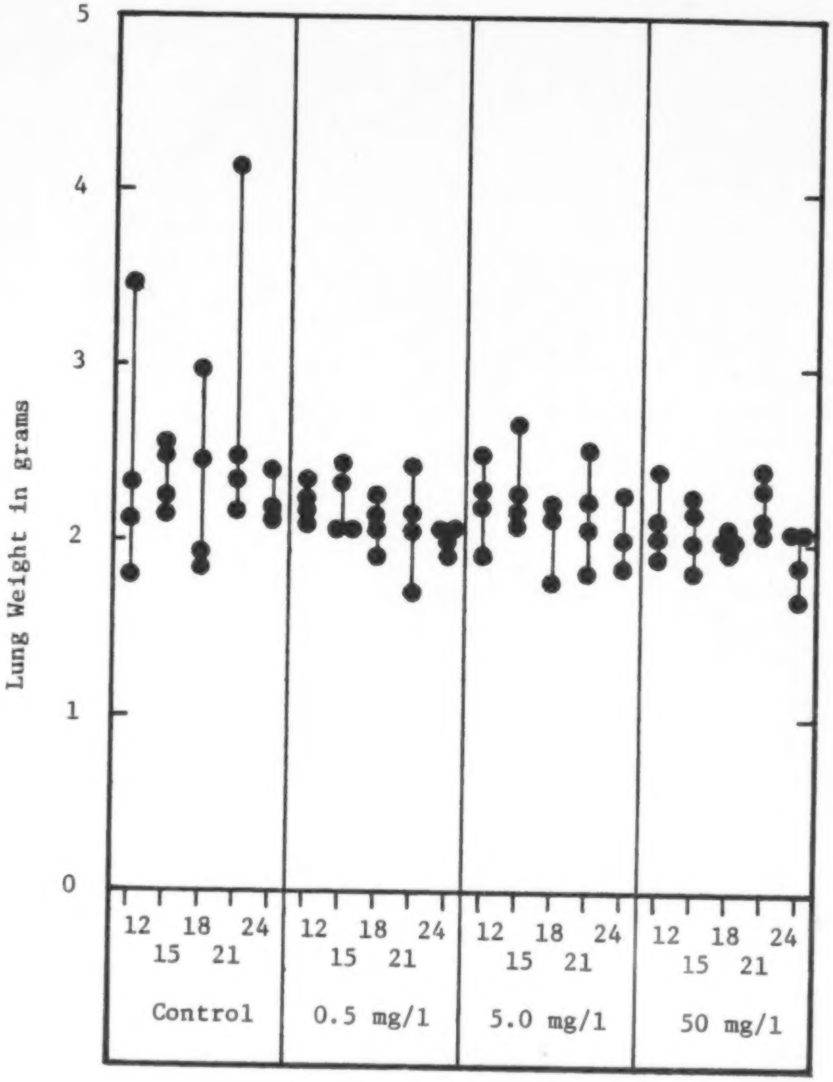

Figure 7-2. Wet lung weight of rats in grams as function of cadium dose and treatment duration.

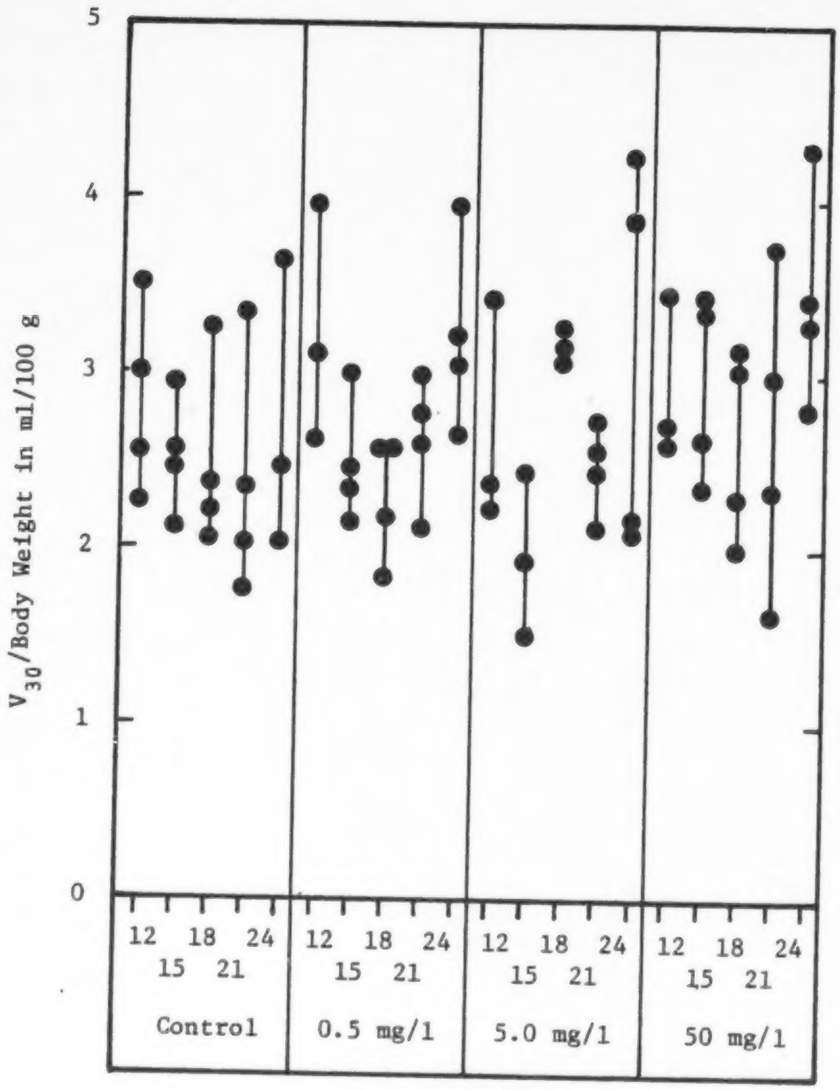

Figure 7-3. Total lung capacity (air lung volume at a transpulmonary pressure of 30 cm water) of rats in ml per 100 grams body weight as function of cadium dose and treatment duration.

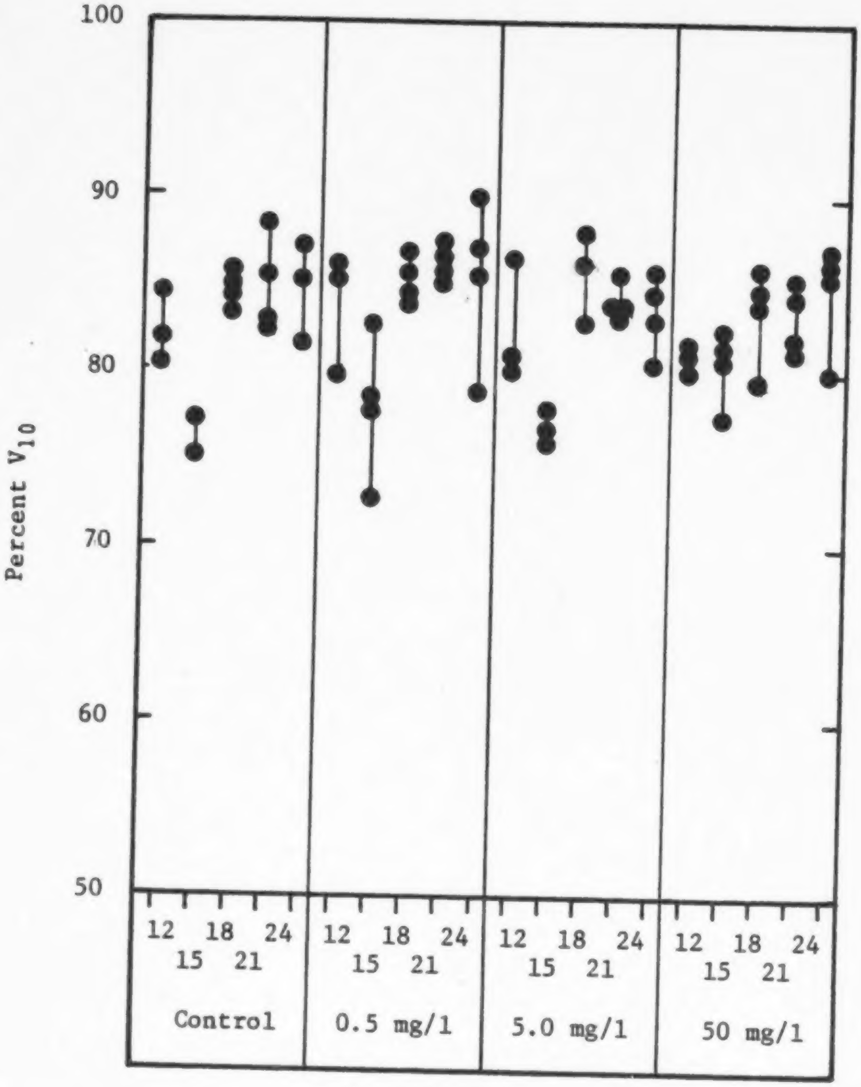

Figure 7-4. Percent lung volume remaining in the lung at transpulmonary pressure of 10 cm water following deflation from 30 cm water as function of cadium dose and treatment duration.

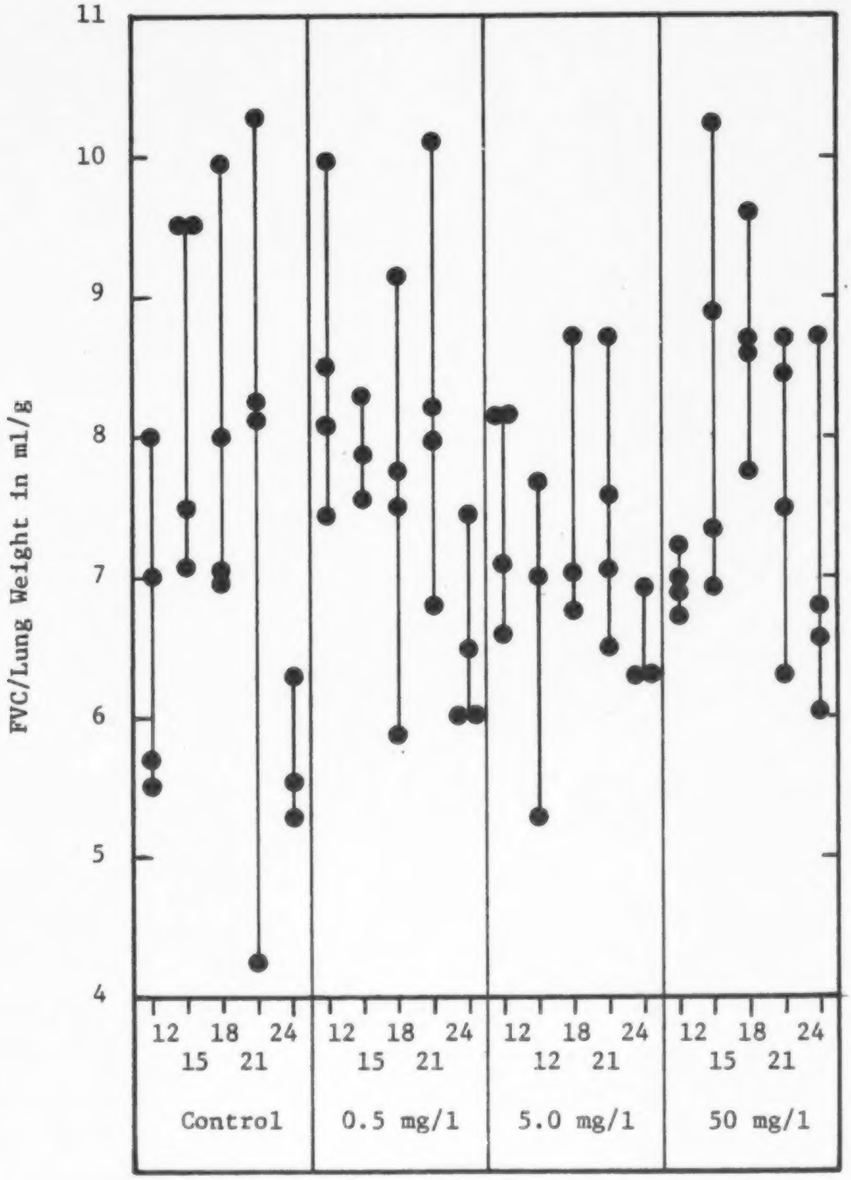

Figure 7-5. Forced vital capacity in ml per gram of lung weight as function of cadmium dose and treatment duration.

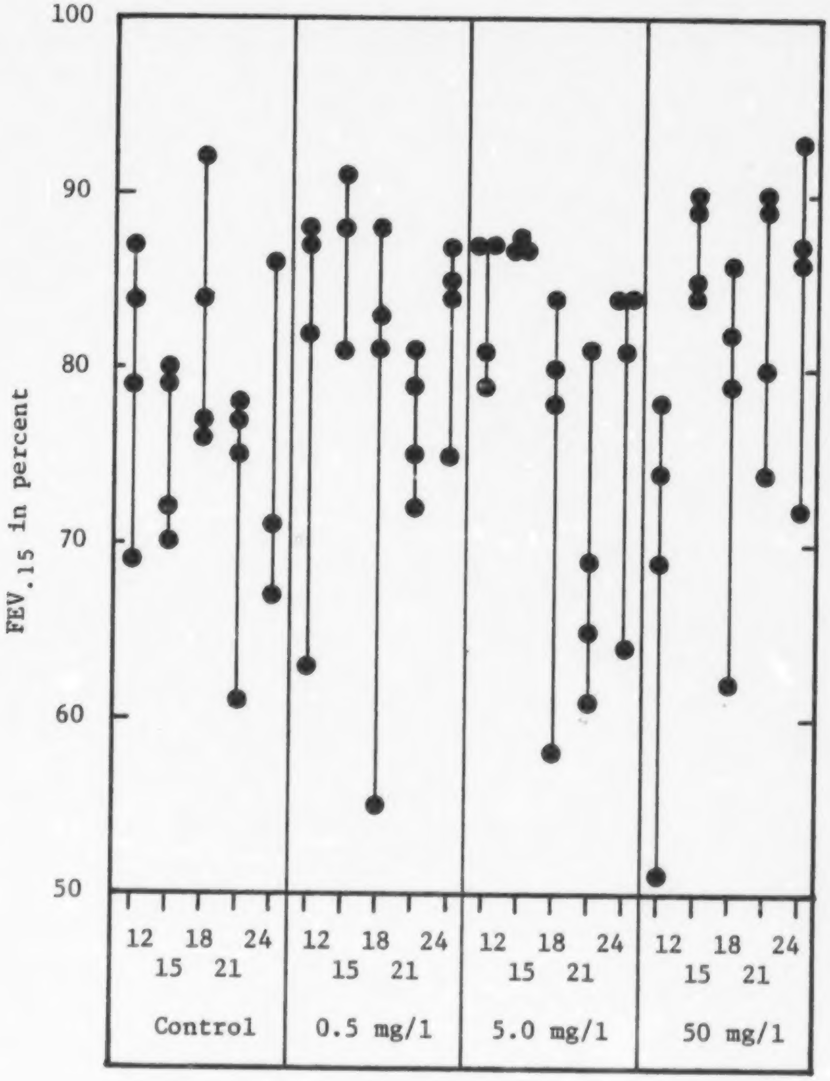

Figure 7-6. Forced expiratory volume at 0.15 seconds in percent of forced vital capacity as function of cadmium dose and treatment duration.

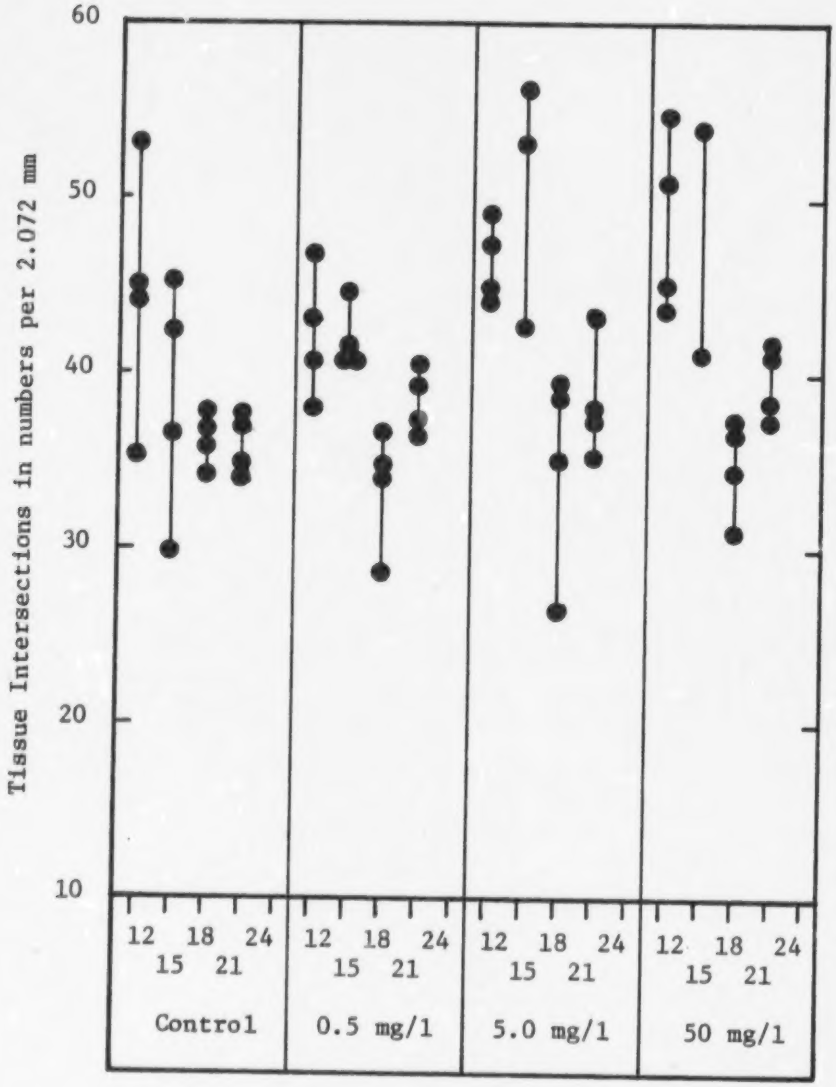

Figure 7-7. Number of intersections with alveolar walls of a randomly placed grid of parallel lines as function of cadmium dose and treatment duration. Length of lines in the grid = 2.072 mm.

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APPENDICIES

Table A-1. Group Mean ± Standard Deviation (S.D.) of Hematocrit (as Percent Packed Red Blood Cells) as a Function of Age and Exposure to Cd

Months	Control	Group 2	Group 3	Group 4
0 mos.	46.40 ± 4.50	46.40 ± 4.50	46.40 ± 4.50	46.40 ± 4.50
3 mos.	68.94 ± 7.39	64.86 ± 9.09	63.59 ± 4.56	61.30 ± 10.61
6 mos.	60.84 ± 4.14	64.16 ± 8.51	62.64 ± 7.36	61.09 ± 8.75
9 mos.	59.42 ± 13.24	62.51 ± 7.91	63.96 ± 8.76	61.06 ± 11.17
12 mos.	63.46 ± 8.99	60.97 ± 5.88	62.03 ± 6.47	55.42 ± 9.67
15 mos.	66.41 ± $11.36^{a,b}$	53.76 ± 6.16^{a}	56.64 ± 6.63	53.94 ± 2.44^{b}
18 mos.	56.48 ± 10.26	64.08 ± 6.24	57.79 ± 9.23	54.70 ± 18.48
21 mos.	66.59 ± 18.83	68.32 ± 15.95	75.76 ± 8.37	59.68 ± 11.13
24 mos.	56.10 ± 12.11	54.18 ± 7.08	59.16 ± 9.71	51.19 ± 6.77
Overall	62.27^{b}	61.84	62.82^{a}	$57.00^{a,b}$

At each time point, the mean for a particular exposure group was obtained from the data of 6 to 8 rats.

The table is read from left to right so that the mean values of the different exposure groups can be compared at each time point.

The "overall" mean for each test group was calculated from the data of all the rats within the group from 0 through 24 months. This value represents the mean of 54 to 72 rats.

A one-way ANOVA was performed at each time point to determine significant differences between the individual group means. A two-way ANOVA was performed on the "overall" means to test for significant differences between the exposure groups. In the two-way ANOVA, an adjustment was made for age effects.

At each time point, group mean values that are significantly different from each other are given the same superscript. The superscripts "a" and "b" denote that the difference between these group means is significant at $p \leq 0.05$.

Table A-2. Mean Fecal Cadmium (µg Cd/gram dried feces) as a Function of Age and Exposure to Cd

Months	Control	Group 2	Group 3	Group 4
0	0.96	0.96	0.96	0.96
3	1.95[a]	6.12[b]	45.04[a,b]	176.86[a,b]
6	2.77[a]	8.87[b]	40.34[c]	337.40[a,b,c]
9	0.71[a]	6.62[b]	46.73[a,b]	297.18[a,b]
12	0.99[a,x]	5.71[b,x]	48.31[c,x]	309.99[a,b,c]
15	ND	ND	ND	ND
18	0.51[a]	4.53[b]	51.17[c]	443.35[a,b,c]
21	ND	ND	ND	ND
24	0.47[a]	6.30[b]	59.02[c]	462.69[a,b,c]
Overall	1.26[a,d]	6.40[b,x]	47.89[c,d,x]	335.67[a,b,c]

[a,b,c,d] Denote that the differences between group means are significant at $p \leq 0.05$.

[x] Denotes that the difference between group means is significant at $p = 0.100$.

ND = no data.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-3. The Effects That Early vs. Late Arrival and Dosage Groups Have on Rat Urine, Tissue and Fecal Variables

Months:	0	3	6	9	12	15	18	21	24	Overall
Hematocrit										
Early vs. Late	o	o	o	*	o	o	*	o	o	o
Group	NA	o	o	o	o	*	o	o	o	*
Liver weight (g)										
Early vs. Late	*(0.094)	o	o	*	o	o	o	o	o	*
Group	NA	o	o	o	o	*	o	o	o	o
Liver weight (% body weight)										
Early vs. Late	o	*	*(0.072)	*(0.062)	o	o	o	o	*	*
Group	NA	o	o	o	o	o	o	o	o	o
Liver-wet/dry weight										
Early vs. Late	*	o	o	o	*	o	o	o	*	*(0.056)
Group	NA	o	o	o	o	o	o	o	o	o
Liver-μg Cd/gram dry weight										
Early vs. Late	*	o	o	*	*	*	o	o	o	o
Group	NA	*	*	*	*	*	*	*	*	*
Liver-μg Zn/gram dry weight										
Early vs. Late	*(0.061)	*	o	o	*	o	o	o	o	*
Group	NA	*	*(0.068)	*	*	*	*	*	*	*
Liver-μg Cu/gram dry weight										
Early vs. Late	o	o	*	o	*	o	o	o	o	o
Group	NA	o	o	o	o	o	o	o	*	*
Lung weight (gram)										
Early vs. Late	*(0.075)	*	*	o	o	o	*	*	o	*
Group	NA	o	o	o	o	o	o	o	*	o
Lung weight (% body weight)										
Early vs. Late	*	*	*	o	o	o	o	*	*	*
Group	NA	*	o	o	o	o	o	o	o	o
Lung-wet/dry weight										
Early vs. Late	*	o	*	o	o	o	o	o	o	*
Group	NA	o	o	o	o	o	o	o	o	o
Lung-μg Cd/gram dry weight										
Early vs. Late	o	o	o	o	o	o	o	o	o	o
Group	NA	o	*	*	*	*	*	*	*	*
Lung-μg Zn/gram dry weight										
Early vs. Late	o	*	*(0.076)	o	o	o	*	o	o	*
Group	NA	o	o	o	*	o	o	o	o	*(0.073)
Lung-μg Cu/gram dry weight										
Early vs. Late	o	o	o	o	o	o	o	o	o	o
Group	NA	o	o	o	o	o	o	o	o	o
Muscle-μg Cd/gram dry weight										
Early vs. Late	*	o	*	o	*(0.085)	o	ND	ND	*	o
Group	NA	o	*(0.086)	o	o	o	ND	ND	*	o
Muscle-μg Zn/gram dry weight										
Early vs. Late	*	*	o	o	o	o	ND	ND	o	*
Group	NA	o	o	o	o	o	ND	ND	*	o
Muscle-μg Cu/gram dry weight										
Early vs. Late	o	o	o	o	o	o	ND	ND	o	o
Group	NA	*	o	o	o	o	ND	ND	o	o
Intestine-weight (gram)										
Early vs. Late	o	o	*	*	*	*	*	*	o	*
Group	NA	o	o	o	o	o	o	o	o	o

Table A-3

Continued

Months:	0	3	6	9	12	15	18	21	24	Overall
Intestine-% body weight										
Early vs. Late	*	□	*(0.062)	o	o	*	*	*	*	*
Group	NA	*(0.060)	□	*(0.075)	o	o	o	o	o	o
Intestine-wet/dry weight										
Early vs. Late	*	o	o	o	o	o	o	*	o	o
Group	NA	o	o	o	o	o	o	o	o	o
Intestine-µg Cd/gram dry weight										
Early vs. Late	*	o	o	*	*	o	*	o	o	*
Group	NA	*	*	*	*	*	*	*	*	*
Intestine-µg Zn/gram dry weight										
Early vs. Late	*	*	o	o	o	*	o	*	*	o
Group	NA	o	o	o	o	o	o	o	o	o
Intestine-µg Cu/gram dry weight										
Early vs. Late	*	o	o	o	o	o	o	o	o	o
Group	NA	o	o	o	o	o	o	o	o	o
Left kidney-weight (g)										
Early vs. Late	o	*(0.077)	o	o	*	*(0.080)	*	o	*	*
Group	NA	o	o	o	o	*(0.088)	*	o	o	o
Left kidney-% body weight										
Early vs. Late	*	*	o	o	*	*	o	o	*	*
Group	NA	*	*	*	*	*	*	o	o	*
Kidney-wet dry weight										
Early vs. Late	*	o	o	*	o	o	o	o	*(0.056)	o
Group	NA	o	o	o	o	o	o	o	□	o
Kidney-µg Cd/gram dry weight										
Early vs. Late	*	o	*	o	o	o	o	*	o	o
Group	NA	*	*	*	*	*	*	*	*	*
Kidney-µg Zn/gram dry weight										
Early vs. Late	*	o	*	o	o	o	o	o	o	o
Group	NA	*	*	*	*	*	*	*	*	*
Kidney-µg Cu/gram dry weight										
Early vs. Late	*	o	o	o	o	o	*	*	*	*
Group	NA	o	*	*	*	*	*	*	*	*
Right kidney-weight (gram)										
Early vs. Late	*(0.077)	*(0.073)	o	o	*	*(0.054)	*	*(0.070)	*(0.069)	*
Group	NA	o	o	o	o	□	*	o	□	o
Right kidney-% body weight										
Early vs. Late	o	*	o	o	*	*	o	o	*	*
Group	NA	o	o	*	*	*	*	o	o	*
Renal LAP										
Early vs. Late	ND	ND	o	*	o	o	*	*	*	o
Group	NA	ND	o	o	o	o	*	o	o	o
Tubules-µg Cd/gram dry weight										
Early vs. Late	o	o	o	o	o	*	o	o	o	o
Group	NA	*	*	*	*	*	*	*	*	*
Tubules-µg Zn/gram dry weight										
Early vs. Late	*	o	o	o	o	*	o	*	o	o
Group	NA	o	o	*(0.096)	*	*	o	*	o	*
Tubules-µg Cu/gram dry weight										
Early vs. Late	*	o	o	o	o	o	*	*	o	o
Group	NA	*(0.087)	o	*	*	*	*	*	*	*

Table A-3 Continued

Months:	0	3	6	9	12	15	18	21	24	Overall
Urine volume - ml/24 hrs										
Early vs. Late	o	o	*	o	o	*	o	o	o	*
Group	NA	*	*	o	o	*	o	*	o	*
Urine pH										
Early vs. Late	o	o	o	*	*	o	o	o	o	*
Group	NA	*	o	*	*	o	*	o	o	*
Urine-specific gravity										
Early vs. Late	o	o	*	o	o	o	o	o	o	o
Group	NA	*	*	*	*	*	*(0.070)	*	o	*
Urine-total protein (mg/day)										
Early vs. Late	*	*	o	*	*	*	*	*	*	*
Group	NA	*	o	o	o	o	o	o	o	o
Urine-LAP (s.u./day)										
Early vs. Late	*(0.096)	*	ND	*	*	*	*	*	o	*
Group	NA	o	ND	o	o	o	o	o	o	o
Urine-glucose (mg/day)										
Early vs. Late	o	ND	ND	*	o	o	o	o	o	o
Group	NA	ND	ND	o	o	o	o	o	o	o
Feces-µg Cd/gram dry weight										
Early vs. Late	*	*	o	o	*	ND	*	ND	o	o
Group	NA	*	*	*	*	ND	*	ND	*	*
Feces-µg Zn/gram dry weight										
Early vs. Late	*	*	*(0.080)	*	o	ND	*	ND	*(0.065)	*
Group	NA	o	*	o	o	ND	o	ND	o	o
Feces-µg Cu/gram dry weight										
Early vs. Late	*	*	o	*	o	ND	*	ND	o	o
Group	NA	o	o	o	o	ND	o	ND	o	o

"Early vs. Late" indicates the results of the analysis of variance (ANOVA) performed for each variable at each time point to determine if there was a significant difference between the early and late arriving rats, irrespective of the effects due to Cd exposure. For this analysis, an adjustment was made for the differences due to Cd exposure.

"Group" indicates the results of the ANOVA performed for each variable at each time point to determine if there was a significant effect due to Cd exposure, regardless of whether the rats belonged to the early or late arriving group. For this analysis, an adjustment was made for differences due to early vs. late arrival.

For each variable, at each time point, the mean values for the Control, Group 2, Group 3 and Group 4 are compared with each other. Within each group, at each time point, the data from 6 to 8 rats were used.

*	Differences between group means are significant at $p \leq 0.05$.
*(z)	Differences between group means are significant at $p = z$.
o	Differences between group means are not significant.
NA	Not applicable.
ND	No data.

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Table A-4. Mean Concentration of Cadmium in the Renal Cortex (μg Cd/gram dry cortex) as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	1.52	1.52	1.52	1.52
3	3.64^{c}	1.88^{b}	13.29^{a}	$136.4^{a,b,c}$
6	0.00^{c}	3.96^{b}	17.81^{a}	$169.8^{a,b,c}$
9	0.00^{c}	3.16^{b}	28.21^{a}	$306.2^{a,b,c}$
12	0.94^{c}	5.33^{b}	41.74^{a}	$417.8^{a,b,c}$
15	$2.97^{c,x}$	13.03^{b}	$61.76^{a,x}$	$467.1^{a,b,c}$
18	$0.19^{c,x}$	3.16^{b}	$45.91^{a,x}$	$444.2^{a,b,c}$
21	0.41^{c}	2.44^{b}	49.00^{a}	$432.0^{a,b,c}$
24	0.11^{c}	1.65^{b}	40.06^{a}	$374.5^{a,b,c}$
Overall	$1.06^{c,d}$	4.51^{b}	$38.43^{a,d}$	$352.6^{a,b,c}$

^{a,b,c,d}Denote that the differences between group means are significant at $p \leq 0.05$.

^{x,y,z}Denote that the differences between group means are significant at $p = 0.100$.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-5. Mean Cortical Tubular Cadmium (μg/Cd/g dried cortical tubules) as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	1.96	1.96	1.96	1.96
3	2.71[a]	12.36[b]	39.51[c]	179.45[a,b,c]
6	1.65[a]	6.62[b]	29.86[c]	205.11[a,b,c]
9	1.35[a]	6.92[b]	36.49[c]	370.80[a,b,c]
12	2.74[a]	10.32[b]	60.47[c]	483.26[a,b,c]
15	8.49[a,d]	57.19[b,e]	146.63[c,d,e]	634.41[a,b,c]
18	1.88[a]	28.85[b]	69.04[c]	572.80[a,b,c]
21	6.84[a]	13.38[b]	41.03[c]	333.02[a,b,c]
24	4.66[a]	7.24[b]	20.74[c]	275.63[a,b,c]
Overall	3.92[a,d]	18.01[b]	55.58[c,d]	370.13[a,b,c]

[a,b,c,d,e] Denote that the differences between group means are significant at $p \leq 0.05$.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-6. Mean Renal Copper (μg Cu/g dry cortex) as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	32.50	32.50	32.50	32.50
3	41.76	36.79	37.66	49.70
6	46.74[a]	47.46[b]	43.66[c]	72.10[a,b,c]
9	45.37[a]	43.87[b]	47.76[c]	96.28[a,b,c]
12	43.54[a]	36.77[b]	56.81[c]	92.00[a,b,c]
15	42.59[a]	48.35[b]	69.32[c]	122.46[a,b,c]
18	29.23[a]	34.17	52.04	66.41[a]
21	35.74	49.64	50.44	78.64
24	40.32[a]	34.33[b]	42.53[c]	82.59[a,b,c]
Overall	41.68[a]	42.11[b]	50.01[c]	79.59[a,b,c]

[a,b,c] Denote that the differences between group means are significant at $p \leq 0.05$.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-7. Mean Cortical Tubular Copper (µg Cu/g dry cortical tubules) as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	58.70	58.70	58.70	58.70
3	53.48	49.49[x]	59.17	85.11[x]
6	60.92	68.98	53.37[x]	91.50[x]
9	61.20[a]	60.32[b]	76.43[c]	145.88[a,b,c]
12	55.14[a]	49.44[b]	72.76[c]	143.40[a,b,c]
15	60.53[a]	86.57[x]	122.21	198.56[a,x]
18	55.18	58.07	70.78	107.94
21	36.18[a]	61.84	44.74	70.71[a]
24	36.61	26.99	19.22[x]	43.77[x]
Overall	51.90[a]	57.39[b]	67.47[c]	101.35[a,b,c]

[a,b,c] Denote that the differences between group means are significant at $p \leq 0.05$.

[x] Denotes that the differences between group means are significant at $p = 0.100$.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-8. Mean Renal Zinc (μg Zn/g dry cortex) as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	116.11	116.11	116.11	116.11
3	130.80	119.00[a]	195.00[a]	171.70
6	87.00	91.59	102.69	118.16
9	90.52[c]	85.53[b]	96.57[a]	126.19[a,b,c]
12	103.77	97.01[x]	114.10	136.49[x]
15	107.19[a]	101.77[b,x]	129.35[x]	144.57[a,b]
18	88.52[c]	96.56[b]	113.99[a]	137.10[a,b,c]
21	82.23[c,d]	87.78[b,e]	111.11[a,d,e]	128.74[a,b,c]
24	105.50	94.46[a]	113.99	129.08[a]
Overall	99.45[c,d]	97.09[a,b]	121.64[a,c]	138.48[b,d]

[a,b,c,d,e] Denote that the differences between group means are significant at $p \leq 0.05$.

[x,y,z] Denote that the differences between group means are significant at $p = 0.100$.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-9. Mean Cortical Tubular Zinc (μg Zn/g dry cortical tubules) as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	92.45	92.45	92.45	92.45
3	223.56	254.19	289.24	317.00
6	155.51	155.90	169.64	188.01
9	135.04	137.44	157.60	228.50
12	137.38[a]	121.90[b]	194.98[c]	290.40[a,b,c]
15	243.70[x]	267.05	320.70	325.60[x]
18	230.55	215.77	239.79	351.90
21	117.56	108.53[x]	107.52[a]	154.45[a,x]
24	103.86	84.80	86.51	102.00
Overall	166.68[a]	173.97[b]	187.55[c]	267.21[a,b,c]

[a,b,c] Denote that the differences between group means are significant at $p \leq 0.05$.

[x] Denote that the differences between group means are significant at $p = 0.100$.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-10. Mean Hepatic Cadmium (µg Cd/g dry tissue) as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	0.28	0.28	0.28	0.28
3	1.36[a]	2.16[b]	6.34[c]	40.10[a,b,c]
6	0.00[a]	0.35[b]	4.68[c]	46.35[a,b,c]
9	0.08[a]	0.11[b]	2.68[c]	50.60[a,b,c]
12	0.10[a]	0.19[b]	3.02[c]	57.88[a,b,c]
15	0.03[a]	0.22[b]	3.11[c]	77.14[a,b,c]
18	0.00[a]	0.06[b]	2.37[c]	73.13[a,b,c]
21	0.14[a]	0.21[b]	4.37[c]	91.04[a,b,c]
24	0.01[a]	0.22[b]	5.84[c]	112.98[a,b,c]
Overall	0.22[a]	0.47[b]	4.02[c]	69.59[a,b,c]

[a,b,c] Denote that the differences between group means are significant at $p \leq 0.05$.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-11. Mean Hepatic Zinc (μg Zn/g dry tissue) as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	67.69	67.69	67.69	67.69
3	103.45[a]	126.89	117.65	137.32[a]
6	92.52	98.63	85.72[x]	113.50[x]
9	79.23[a]	83.44[b]	76.85[c]	102.35[a,b,c]
12	88.96[a]	96.52	93.45[x]	120.58[a,x]
15	89.67[a]	89.19[b]	99.32[c]	131.85[a,b,c]
18	96.19[a]	92.60[b]	88.32[c]	133.86[a,b,c]
21	73.66[a]	83.36[b]	84.01[c]	132.15[a,b,c]
24	100.61[a]	96.68[b]	92.59[c]	137.15[a,b,c]
Overall	90.51[a]	96.12[b]	92.50[c]	125.09[a,b,c]

[a,b,c] Denote that the differences between group means are significant at $p \leq 0.05$.

[x] Denotes that the differences between group means are significant at $p = 0.100$.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-12. Mean Hepatic Copper (μg Cu/g dry tissue) as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	10.95	10.95	10.95	10.95
3	10.59	12.82	12.08	12.67
6	11.51	10.42	10.48	11.64
9	11.63	12.06	11.31	12.35
12	12.57	12.32	12.83	12.03
15	12.69	12.86	13.53	15.11
18	13.28	13.32	12.23	13.33
21	12.08	12.70	12.07	13.18
24	14.59	13.52	12.18[a]	15.40[a]
Overall	12.23	12.45	12.07[x]	13.15[x]

[a] Denotes that the difference between group means is significant at $p \leq 0.05$.

[x] Denotes that the difference between group means is significant at $p = 0.01$.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-13. Mean Concentration of Cadmium (μg/Cd/g dry tissue) in the Intestine as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	0.28	0.28	0.28	0.28
3	1.03^{x}	1.12^{y}	2.82	$6.95^{x,y}$
6	0.16^{a}	0.00^{b}	2.33^{c}	$9.93^{a,b,c}$
9	0.06^{a}	0.08^{b}	1.27^{c}	$10.16^{a,b,c}$
12	0.14^{a}	0.61^{b}	1.68^{c}	$11.59^{a,b,c}$
15	0.05^{a}	0.28^{b}	2.29^{c}	$12.87^{a,b,c}$
18	0.00^{a}	0.18^{b}	1.01^{c}	$10.23^{a,b,c}$
21	0.29^{a}	0.98^{b}	1.80^{c}	$12.01^{a,b,c}$
24	0.01^{a}	0.17^{b}	1.89^{c}	$14.74^{a,b,c}$
Overall	0.31^{a}	0.43^{b}	1.90^{c}	$11.52^{a,b,c}$

[a,b,c] Denote that the differences between group means are significant at $p \leq 0.05$.

[x,y] Denote that the differences between group means are significant at $p = 0.100$

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-14. Mean Concentration of Zinc (μg Zn/g dry tissue) in the Intestine as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	109.72	109.72	109.72	109.72
3	116.41	96.95	102.18	98.58
6	88.75	81.28	105.80	94.09
9	95.50	91.07	75.42	83.36
12	84.87	109.02	114.84	91.13
15	119.62	120.51	97.44	110.98
18	103.32	110.25	92.13	113.29
21	112.62	100.07	107.13	97.30
24	103.72	100.26	101.17	91.12
Overall	103.10	101.18	99.52	97.48

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-15. Mean Concentration of Copper (μg Cu/g dry tissue) in the Intestine as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	8.79	8.79	8.79	8.79
3	4.57	4.69	4.84	5.28
6	4.94	4.18	4.98	4.08
9	4.97	5.07	4.38	3.66
12	4.11	5.17	5.98	5.81
15	5.70	4.99	4.98	5.53
18	4.66	5.33	4.61	4.89
21	5.24	4.55	4.96	4.68
24	4.09	3.99	4.44	3.68
Overall	4.79	4.75	4.90	4.70

There were no significant differences ($p \leq 0.05$) between the group means at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-16. Mean Concentration of Cadmium (µg Cd/g dry tissue) in the Lung as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	0.36	0.36	0.36	0.36
3	0.00	0.00	0.57	1.09
6	0.00[a]	0.00[b]	0.52	1.56[a,b]
9	0.00[a]	0.09[b]	0.41[c]	1.70[a,b,c]
12	0.21[a]	0.26[b]	0.38[c]	1.76[a,b,c]
15	0.23[a]	0.64[x]	0.33[b]	1.80[a,b,x]
18	0.01[a]	0.25[b]	0.00[c]	1.84[a,b,c]
21	0.14[a]	0.30[b]	0.50[c]	1.86[a,b,c]
24	0.00[a]	0.00[b]	0.26[c]	2.19[a,b,c]
Overall	0.07[a,x]	0.22[b]	0.44[c,x]	1.73[a,b,c]

[a,b,c] Denote that the differences between group means are significant at $p \leq 0.05$.

[x] Denotes that the differences between group means are significant at $p = 0.100$.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-17. Mean Concentration of Zinc (μg Zn/g dry tissue) in the Lung as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	90.99	90.99	90.99	90.99
3	80.13	82.44	97.50	89.40
6	75.30	75.40	76.11	78.31
9	66.47	65.72	66.08	66.67
12	69.86	75.86	83.07	71.59
15	77.74	75.39	83.69	73.51
18	70.78	67.35	75.22	70.12
21	67.40	67.61	64.93	64.73
24	75.21	72.58	71.33	73.82
Overall	72.86	72.82	77.24	73.52

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-18. Mean Concentration of Copper (μg Cu/g dry tissue) in the Lung as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	9.15	9.15	9.15	9.15
3	6.16	6.33	5.98	6.56
6	6.51	5.60	6.50	6.68
9	5.65	5.95	5.94	5.65
12	6.72	7.34	7.48	6.41
15	6.65	5.08	6.00	6.19
18	6.04	5.78	6.61	6.28
21	6.21	5.86	5.97	5.56
24	7.08	5.35	5.82	5.97
Overall	6.38	5.91	6.29	6.16

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-19. Mean Concentration of Cadmium (μg Cd/g dry tissue) in the Muscle as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00
6	0.00	0.00	0.32	0.20
9	0.00	0.00	0.00	0.00
12	0.00	0.00	0.14	0.24
15	0.00	0.16	0.08	0.19
18	ND	ND	ND	ND
21	ND	ND	ND	ND
24	0.00	0.03	0.00	0.31
Overall	0.00	0.03	0.09	0.15

ND = No data.

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-20. Mean Concentration of Zinc (µg Zn/g dry tissue) in the Muscle as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	45.77	45.77	45.77	45.77
3	60.74	63.49	61.43	58.74
6	50.66	49.13	50.95	53.38
9	44.02	45.09	46.35	48.23
12	45.06	46.48	54.82	60.77
15	58.50	56.16	57.60	56.28
18	ND	ND	ND	ND
21	ND	ND	ND	ND
24	68.02	55.95	54.93	56.48
Overall	54.50	52.75	54.35	55.65

ND = No data.

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-21. Mean Concentration of Copper (µg Cu/g dry tissue) in the Muscle as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	5.47	5.47	5.47	5.47
3	4.04	3.78	3.99	2.62
6	2.86	3.04	3.35	2.75
9	3.40	3.15	2.99	2.98
12	3.79	3.91	3.61	3.88
15	3.41	3.52	3.28	3.56
18	ND	ND	ND	ND
21	ND	ND	ND	ND
24	3.05	2.88	3.17	3.26
Overall	3.43	3.38	3.23	3.18

ND = No data.

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-22. Mean Concentration of Cadmium (µg Cd/g dry tissue) in the Heart as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	ND	ND	ND	ND
3	0.00[a]	0.00[b]	0.00[c]	1.52[a,b,c]
6	0.00[a]	0.00[b]	0.00[c]	1.18[a,b,c]
9	0.00[x]	0.21	0.23	0.87[x]
12	0.00[a]	0.20[b]	0.26	1.33[a,b]
15	0.00[a]	0.00[b]	0.00[c]	0.95[a,b,c]
18	ND	ND	ND	ND
21	ND	ND	ND	ND
24	0.00[a]	0.00[b]	0.09[c]	1.36[a,b,c]
Overall	0.01[a]	0.07[b]	0.09[c]	1.20[a,b,c]

[a,b,c] Denote that the differences between group means are significant at $p \leq 0.05$.

[x] Denotes that the differences between group means are significant at $p = 0.100$.

ND = No data.

See Table A-1 footnotes for an explanation of table construction and data analysis.

At each time point, the mean for a particular exposure group was obtained from the data of 4 rats. The "overall" mean for each group was calculated from the data for 24 rats.

Table A-23. Mean Concentration of Zinc (µg Zn/g dry tissue) in the Heart as a Function of Age and Exposure of Cadmium

Months	Control	Group 2	Group 3	Group 4
0	ND	ND	ND	ND
3	44.60	43.90	42.80	60.93
6	64.78	67.25	67.90	67.37
9	53.43	55.76	54.97	54.83
12	50.85	65.40	53.90	62.22
15	65.88	64.62	64.23	63.00
18	ND	ND	ND	ND
21	ND	ND	ND	ND
24	71.29	69.42	66.20	63.75
Overall	58.47	61.01	58.33	62.02

ND = No data.

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

At each time point, the mean for a particular exposure group was obtained from the data of 4 rats. The "overall" mean for each group was calculated from the data for 24 rats.

Table A-24. Mean Concentration of Copper (μg Cu/g dry tissue) in the Heart as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	ND	ND	ND	ND
3	12.49	12.90	12.42	15.48
6	21.08	18.78	20.33	19.18
9	20.05	20.37	19.77	19.28
12	17.21	17.93	17.95	18.01
15	21.42	23.06	22.26	22.27
18	ND	ND	ND	ND
21	ND	ND	ND	ND
24	18.28	19.67	20.46	18.49
Overall	18.42	18.79	18.87	18.79

ND = No data.

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

At each time point, the mean for a particular exposure group was obtained from the data of 4 rats. The "overall" mean for each group was calculated from the data for 24 rats.

Table A-25. Concentration of Cadmium (μg Cd/g dry tissue) in the Spleen as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	ND	ND	ND	ND
3	ND	ND	ND	ND
6	0.00^{a}	0.80^{b}	0.36^{c}	$5.13^{a,b,c}$
9	0.00^{a}	0.00^{b}	0.38^{c}	$3.14^{a,b,c}$
12	0.00^{a}	0.25^{b}	0.63^{c}	$6.45^{a,b,c}$
15	0.00^{a}	0.16^{b}	0.21^{c}	$4.65^{a,b,c}$
18	ND	ND	ND	ND
21	ND	ND	ND	ND
24	0.12^{a}	0.00^{b}	0.11^{c}	$8.94^{a,b,c}$
Overall	0.02^{a}	0.20^{b}	0.28^{c}	$5.45^{a,b,c}$

^{a,b,c}Denote significant differences between group means of $p \leq 0.05$.

ND = No data.

See Table A-1 footnotes for an explanation of table construction and data analysis.

At each time point, the mean for a particular exposure group was obtained from the data of 4 rats. The "overall" mean for each group was calculated from the data for 20 rats.

Table A-26. Concentration of Zinc (μg Zn/g dry tissue) in the Spleen as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	ND	ND	ND	ND
3	ND	ND	ND	ND
6	62.97	76.87	78.30	81.13
9	61.64	62.57	62.64	63.92
12	70.69	71.26	74.56	79.28
15	65.15	75.32	69.30	72.57
18	ND	ND	ND	ND
21	ND	ND	ND	ND
24	65.39	74.00	72.32	72.24
Overall	66.80	72.68	71.09	78.78

ND = No data.

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

At each time point, the mean for a particular exposure group was obtained from the data of 4 rats. The "overall" mean for each group was calculated from the data for 20 rats.

Table A-27. Concentration of Copper (µg Cu/g dry tissue) in the Spleen as a Function of Age and Exposure to Cadmium

Months	Control	Group 2	Group 3	Group 4
0	ND	ND	ND	ND
3	ND	ND	ND	ND
6	5.24	5.68	4.64	6.71
9	4.42	5.05	4.87	4.23
12	5.83	6.03	5.81	6.07
15	5.61	5.94	6.52	5.18
18	ND	ND	ND	ND
21	ND	ND	ND	ND
24	5.67	5.73	5.19	5.23
Overall	5.19	5.51	5.33	5.46

ND = No data.

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

At each time point, the mean for a particular exposure group was obtained from the data of 4 rats. The "overall" mean for each group was calculated from the data for 24 rats.

Table A-28. Intestine Wet Weight, as Group Mean (g) ± Standard Deviation as a Function of Age and Cd Exposure

Months	Control	Group 2	Group 3	Group 4
0	7.33 ± 0.67	7.33 ± 0.67	7.33 ± 0.67	7.33 ± 0.67
3	6.07 ± 2.46	6.02 ± 1.20	5.11 ± 0.37	6.20 ± 1.31
6	5.82 ± 1.15	5.02 ± 0.62	5.26 ± 0.86	5.02 ± 0.83
9	4.79 ± 0.62	4.84 ± 0.88	4.96 ± 0.82	4.79 ± 0.62
12	4.01 ± 0.64	4.51 ± 0.77	4.49 ± 0.95	4.06 ± 0.73
15	5.11 ± 1.22	4.80 ± 1.18	4.26 ± 1.03	4.20 ± 1.89
18	4.92 ± 1.26	4.29 ± 0.91	4.84 ± 1.55	5.09 ± 3.62
21	5.93 ± 1.00	5.98 ± 1.02	5.41 ± 1.26	6.14 ± 1.44
24	4.93 ± 1.09	5.23 ± 0.77	4.91 ± 0.77	4.95 ± 0.87

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-29. Lung Wet Weight, as Group Mean (g) ± Standard Deviation as a Function of Age and Cd Exposure

Months	Control	Group 2	Group 3	Group 4
0	1.04 ± 0.11	1.04 ± 1.11	1.04 ± 0.11	1.04 ± 0.11
3	1.48 ± 0.14	1.40 ± 0.11	1.38 ± 0.11	1.42 ± 0.11
6	1.46 ± 0.18	1.41 ± 0.21	1.45 ± 0.14	1.43 ± 0.13
9	1.70 ± 0.32	1.60 ± 0.25	1.60 ± 0.17	1.45 ± 0.10
12	2.08 ± 0.68	1.92 ± 0.35	2.00 ± 0.35	1.85 ± 0.33
15	2.06 ± 0.40	1.99 ± 0.29	2.02 ± 0.47	1.88 ± 0.27
18	1.99 ± 0.32	1.84 ± 0.35	1.96 ± 0.22	2.02 ± 0.17
21	2.36 ± 0.88	1.94 ± 0.32	1.95 ± 0.33	2.04 ± 0.29
24	2.16 ± 0.29	2.04 ± 0.09	1.97 ± 0.20	1.86 ± 0.17

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-30. Heart Wet Weight, as Group Mean (g) ± Standard Deviation as a Function of Age and Cd Exposure

Months	Control	Group 2	Group 3	Group 4
0	ND	ND	ND	ND
3	1.32[1]	ND	1.49[1]	1.25[1]
6	1.35 ± 0.09	1.51 ± 0.44	1.38 ± 0.16	1.39 ± 0.19
9	1.52 ± 0.06	1.40 ± 0.26	1.33 ± 0.11	1.29 ± 0.39
12	1.42 ± 0.10	1.54 ± 0.12	1.44 ± 0.17	1.37 ± 0.14
15	1.55 ± 0.16	1.58 ± 0.08	1.44 ± 0.22	1.56 ± 0.11
18	1.93 ± 0.50	1.54 ± 0.15	1.64 ± 0.31	1.62 ± 0.23
21	1.70 ± 0.33	1.74 ± 0.11	1.67 ± 0.11	1.75 ± 0.28
24	1.97 ± 0.38	1.78 ± 0.28	1.71 ± 0.13	1.70 ± 0.30

[1] Mean value of 2 rats.

ND = No data.

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-31. Spleen Wet Weight, as Group Mean (g) ± Standard Deviation as a Function of Age and Cd Exposure

Months	Control	Group 2	Group 3	Group 4
0	ND	ND	ND	ND
3	1.00[1]	ND	1.30[1]	0.84[1]
6	1.32 ± 0.11	1.01 ± 0.03	1.17 ± 0.17	1.08 ± 0.24
9	1.20 ± 0.10	1.18 ± 0.08	1.32 ± 0.18	1.20 ± 0.17
12	1.37 ± 0.13	1.21 ± 0.13	1.47 ± 0.35	1.13 ± 0.39
15	1.55 ± 0.28	1.21 ± 0.22	1.96 ± 0.40	1.31 ± 0.34
18	1.64 ± 0.64	1.10 ± 0.22	1.24 ± 0.31	1.13 ± 0.38
21	1.43 ± 0.48	1.39 ± 0.21	1.50 ± 0.45	1.62 ± 0.29
24	1.38 ± 0.54	1.44 ± 0.31	1.18 ± 0.35	1.39 ± 0.54

[1] Mean value of 2 rats.

ND = No data.

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-32. Liver Wet Weight, as Group Mean (g) ± Standard Deviation as a Function of Age and Cd Exposure

Months	Control	Group 2	Group 3	Group 4
0	8.92 ± 1.25	8.92 ± 1.25	8.92 ± 1.25	8.92 ± 1.25
3	18.84 ± 2.03	17.37 ± 3.10	16.98 ± 1.90	16.08 ± 2.20
6	19.55 ± 3.45	18.40 ± 2.50	18.65 ± 3.30	18.24 ± 2.07
9	22.37 ± 3.43	20.43 ± 2.80	22.23 ± 6.49	19.91 ± 3.24
12	18.54 ± 2.62	19.94 ± 3.63	20.34 ± 2.32	18.00 ± 2.78
15	24.57 ± 4.19	22.60 ± 9.26	20.19 ± 2.87	19.37 ± 2.73
18	20.43 ± 2.33	20.39 ± 2.43	21.05 ± 3.58	19.88 ± 5.30
21	23.51 ± 4.48	22.23 ± 2.66	22.73 ± 3.90	22.35 ± 1.27
24	19.31 ± 4.14	18.65 ± 2.64	20.48 ± 3.16	19.54 ± 3.40

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-33. Intestine Wet Weight, as Percent of Body Weight (Group Mean ± Standard Deviation) as a Function of Age and Cd Exposure

Months	Control	Group 2	Group 3	Group 4
0	3.67 ± 0.63	3.67 ± 0.63	3.67 ± 0.63	3.67 ± 0.63
3	1.14 ± 0.18	1.17 ± 0.23	0.99 ± 0.07	1.30 ± 0.30
6	0.95 ± 0.12	0.91 ± 0.10	0.92 ± 0.19	0.89 ± 0.14
9	0.67 ± 0.12	0.61 ± 0.17	0.75 ± 0.06	0.76 ± 0.09
12	0.57 ± 0.10	0.64 ± 0.08	0.57 ± 0.12	0.64 ± 0.11
15	0.65 ± 0.22	0.59 ± 0.14	0.58 ± 0.14	0.63 ± 0.12
18	0.60 ± 0.13	0.54 ± 0.10	0.56 ± 0.13	0.64 ± 0.41
21	0.68 ± 0.15	0.71 ± 0.13	0.65 ± 0.14	0.73 ± 0.17
24	0.69 ± 0.17	0.69 ± 0.11	0.66 ± 0.18	0.76 ± 0.25

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-34. Lung Wet Weight, as Percent of Body Weight (Group Mean ± Standard Deviation) as a Function of Age and Cd Exposure

Months	Control	Group 2	Group 3	Group 4
0	0.52 ± 0.10	0.52 ± 0.10	0.52 ± 0.10	0.52 ± 0.10
3	0.27 ± 0.04	0.27 ± 0.02	0.27 ± 0.02	0.30 ± 0.01
6	0.24 ± 0.02	0.26 ± 0.06	0.25 ± 0.03	0.26 ± 0.03
9	0.24 ± 0.05	0.23 ± 0.03	0.24 ± 0.03	0.23 ± 0.02
12	0.29 ± 0.08	0.27 ± 0.05	0.29 ± 0.07	0.29 ± 0.05
15	0.25 ± 0.04	0.24 ± 0.03	0.27 ± 0.05	0.28 ± 0.05
18	0.24 ± 0.03	0.23 ± 0.03	0.24 ± 0.05	0.26 ± 0.04
21	0.27 ± 0.14	0.23 ± 0.03	0.23 ± 0.03	0.24 ± 0.04
24	0.31 ± 0.09	0.27 ± 0.08	0.25 ± 0.06	0.29 ± 0.07

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-35. Heart Wet Weight, as Percent of Body Weight (Group Mean ± S.D.) as a Function of Age and Cd Exposure

Months	Control	Group 2	Group 3	Group 4
0	ND	ND	ND	ND
3	0.25 ± 0.02	0.26 ± 0.02	0.29 ± 0.05	0.26 ± 0.02
6	0.24 ± 0.02	0.26 ± 0.04	0.24 ± 0.01	0.25 ± 0.01
9	0.20 ± 0.01	0.20 ± 0.02	0.22 ± 0.01	0.22 ± 0.02
12	0.20 ± 0.03	0.22 ± 0.01	0.20 ± 0.01	0.22 ± 0.01
15	0.19 ± 0.03	0.20 ± 0.02	0.20 ± 0.02	0.23 ± 0.02
18	0.23 ± 0.03	0.20 ± 0.01	0.19 ± 0.02	0.21 ± 0.02
21	0.20 ± 0.04	0.21 ± 0.02	0.20 ± 0.01	0.21 ± 0.02
24	0.28 ± 0.07	0.24 ± 0.08	0.23 ± 0.06	0.26 ± 0.08

ND = No data.

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-36. Spleen Wet Weight, as Percent of Body Weight (Group Mean ± S.D.) as a Function of Age and Cd Exposure

Months	Control	Group 2	Group 3	Group 4
0	ND	ND	ND	ND
3	0.19[1]	0.19[1]	0.26[1]	0.19[1]
6	0.21 ± 0.03	0.20 ± 0.03	0.21 ± 0.03	0.19 ± 0.02
9	0.18 ± 0.04	0.17 ± 0.03	0.19 ± 0.03	0.20 ± 0.02
12	0.19 ± 0.02	0.17 ± 0.04	0.18 ± 0.04	0.18 ± 0.06
15	0.20 ± 0.04	0.14 ± 0.02	0.25 ± 0.04	0.20 ± 0.02
18	0.19 ± 0.06	0.14 ± 0.02	0.14 ± 0.02	0.14 ± 0.05
21	0.16 ± 0.05	0.17 ± 0.03	0.18 ± 0.05	0.19 ± 0.03
24	0.19 ± 0.09	0.19 ± 0.06	0.16 ± 0.07	0.21 ± 0.09

[1] Mean value of 2 rats.

ND = No data.

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-37. Liver Wet Weight, as Percent of Body Weight (Group Mean ± S.D.) as a Function of Age and Cd Exposure

Months	Control	Group 2	Group 3	Group 4
0	4.40 ± 0.33	4.40 ± 0.33	4.40 ± 0.33	4.40 ± 0.33
3	3.44 ± 0.32	3.35 ± 0.44	3.29 ± 0.24	3.36 ± 0.29
6	3.21 ± 0.32	3.34 ± 0.22	2.65 ± 1.40	3.22 ± 0.14
9	3.08 ± 0.33	2.91 ± 0.20	3.21 ± 0.54	3.15 ± 0.38
12	2.60 ± 0.29	2.82 ± 0.43	2.60 ± 0.23	2.80 ± 2.28
15	3.09 ± 0.75	3.02 ± 0.46	2.71 ± 0.20	2.89 ± 0.29
18	2.50 ± 0.32	2.56 ± 0.29	2.49 ± 0.51	2.51 ± 0.48
21	2.71 ± 0.81	2.67 ± 0.49	2.76 ± 0.52	2.69 ± 0.38
24	2.70 ± 0.56	2.45 ± 0.41	2.74 ± 0.75	2.92 ± 0.38

There were no significant differences between group means ($p \le 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-38. Intestine Wet/Dry Weight (Group Mean ± S.D.) as a Function of Age and Cd Exposure

Months	Control	Group 2	Group 3	Group 4
0	5.11 ± 1.01	5.11 ± 1.01	5.11 ± 1.01	5.11 ± 1.01
3	4.38 ± 0.60	4.49 ± 0.62	4.33 ± 0.29	4.79 ± 0.64
6	4.68 ± 0.92	4.52 ± 0.41	4.77 ± 0.66	4.48 ± 0.65
9	5.03 ± 0.54	4.83 ± 0.44	4.69 ± 0.45	4.14 ± 0.35
12	5.07 ± 0.81	5.33 ± 0.64	5.43 ± 0.65	5.40 ± 0.51
15	5.59 ± 0.32	5.74 ± 0.36	5.44 ± 0.35	5.55 ± 0.42
18	5.63 ± 0.39	5.86 ± 0.28	5.66 ± 0.35	5.71 ± 0.71
21	5.20 ± 0.71	5.43 ± 0.33	5.74 ± 0.56	5.58 ± 0.72
24	5.45 ± 0.43	5.56 ± 0.77	5.45 ± 0.65	5.54 ± 0.58

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-39. Lung Wet/Dry Weight (Group Mean ± S.D.) as a Function of Age and Cd Exposure

Months	Control	Group 2	Group 3	Group 4
0	4.68 ± 1.09	4.68 ± 1.09	4.68 ± 1.09	4.68 ± 1.09
3	4.91 ± 0.77	4.59 ± 0.18	4.69 ± 0.12	4.80 ± 0.26
6	4.53 ± 0.10	4.60 ± 0.21	4.67 ± 0.28	4.56 ± 0.18
9	4.81 ± 0.32	4.69 ± 0.18	4.61 ± 0.16	4.60 ± 0.07
12	4.86 ± 0.32	4.75 ± 0.25	4.70 ± 0.34	4.61 ± 0.18
15	4.63 ± 0.31	4.56 ± 0.35	4.67 ± 0.37	4.69 ± 0.20
18	4.68 ± 0.35	4.68 ± 0.26	4.66 ± 0.20	4.85 ± 0.63
21	4.32 ± 0.52	4.29 ± 0.27	4.46 ± 0.30	4.51 ± 0.33
24	4.53 ± 0.26	4.62 ± 0.35	4.58 ± 0.61	4.64 ± 0.44

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-40. Heart Wet/Dry Weight (Group Mean ± S.D.) as a Function of Age and Cd Exosure

Months	Control	Group 2	Group 3	Group 4
0	ND	ND	ND	ND
3	4.62 ± 0.09	4.42 ± 0.16	4.64 ± 0.25	4.69 ± 0.15
6	4.72 ± 0.06	4.57 ± 0.15	4.66 ± 0.29	4.83 ± 0.11
9	4.50 ± 0.18	4.52 ± 0.10	4.71 ± 0.15	4.68 ± 0.12
12	4.55 ± 0.18	4.73 ± 0.06	4.55 ± 0.08	4.21 ± 0.81
15	4.65 ± 0.89	4.65 ± 0.14	4.74 ± 0.18	4.71 ± 0.06
18	ND	ND	ND	ND
21	ND	ND	ND	ND
24	4.99 ± 0.22	4.77 ± 0.20	4.79 ± 0.10	4.65 ± 0.12

ND = No data.

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-41. Spleen Wet/Dry Weight (Group Mean ± S.D.) as a Function of Age and Cd Exposure

Months	Control	Group 2	Group 3	Group 4
0	ND	ND	ND	ND
3	4.17[1]	4.04[1]	4.48[1]	4.20[1]
6	4.08 ± 0.02	4.15 ± 0.13	4.16 ± 0.08	4.23 ± 0.08
9	4.12 ± 0.15	4.07 ± 0.07	4.10 ± 0.03	4.29 ± 0.11
12	4.28 ± 0.09	4.32 ± 0.08	4.26 ± 0.13	4.44 ± 0.23
15	4.16 ± 0.20	4.27 ± 0.15	4.16 ± 0.13	4.18 ± 0.08
18	ND	ND	ND	ND
21	ND	ND	ND	ND
24	4.06 ± 0.04	4.20 ± 0.18	4.25 ± 0.13	4.22 ± 0.08
Overall	4.15[a]	4.19	4.18	4.27[a]

[1] Mean of 2 rats.

[a] Denotes that the difference between group means are significant at $p \leq 0.05$.

ND = No data.

See Table A-1 footnotes for an explanation of table construction and data analysis.

The "overall" mean for each group was calculated from the data of 32 to 42 rats.

Table A-42. Liver Wet/Dry Weight (Group Mean ± S.D.) as a Function of Age and Cd Exposure

Months	Control	Group 2	Group 3	Group 4
0	3.31 ± 0.46	3.31 ± 0.46	3.31 ± 0.46	3.31 ± 0.46
3	3.76 ± 0.12	3.95 ± 0.76	3.75 ± 0.14	3.77 ± 0.18
6	3.63 ± 0.14	3.69 ± 0.19	3.78 ± 0.10	3.81 ± 0.18
9	3.55 ± 0.19	3.61 ± 0.14	3.53 ± 0.12	3.72 ± 0.16
12	3.69 ± 0.18	3.76 ± 0.22	3.64 ± 0.09	3.71 ± 0.10
15	3.67 ± 0.28	3.75 ± 0.28	3.66 ± 0.21	3.86 ± 0.14
18	3.64 ± 0.11	3.55 ± 0.25	3.44 ± 0.29	3.59 ± 0.22
21	3.47 ± 0.30	3.66 ± 0.24	3.62 ± 0.21	3.63 ± 0.23
24	3.61 ± 0.61	3.71 ± 0.18	3.64 ± 0.26	3.73 ± 0.18

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-43. Excretion of Urine LAP (Sigma units/24 hrs) in Early Arriving Rats (Group Mean ± S.D.) as a Function of Age and Cd Exposure

Weeks	Control	Group 2	Group 3	Group 4
1	4.67 ± 1.01	4.51 ± 2.03	3.46 ± 1.90	3.45 ± 1.23
5	38.86 ± 14.97	32.60 ± 6.83	20.76 ± 7.88	22.09 ± 7.04
9	33.67 ± 10.35	22.53 ± 5.50	23.89 ± 6.71	28.64 ± 10.09
13	49.33 ± 14.66	37.50 ± 5.69	49.51 ± 5.83	43.66 ± 13.87
19	35.32 ± 7.10	44.19 ± 3.71	49.62 ± 5.78	35.84 ± 10.96
25	51.99 ± 15.57	89.15 ± 31.08	47.84 ± 7.01	63.69 ± 41.29
31	35.95 ± 10.68	56.22 ± 24.69	57.45 ± 19.04	45.95 ± 21.83
37	49.14 ± 9.67	62.64 ± 29.77	62.83 ± 22.16	58.67 ± 25.95
50	34.87 ± 11.73	44.59 ± 20.87	45.48 ± 9.86	43.41 ± 17.73
62	34.08 ± 3.00	51.77 ± 16.21	39.04 ± 12.09	37.65 ± 14.86
70	32.88 ± 7.76	46.26 ± 15.34	34.29 ± 7.96	36.04 ± 11.84
84	29.70 ± 5.99	30.25 ± 9.05	33.44 ± 9.59	25.47 ± 4.50
96	22.99 ± 0.86	21.85 ± 15.60	15.63 ± 2.96	16.95 ± 7.36

See Table A-1 footnotes for an explanation of table construction and data analysis.

Each value represents the mean of the data from 4 rats. There were no significant differences between group means ($p \leq 0.05$) at any time point tested.

Table A-44. Excretion of Urine LAP (Sigma units/24 hours) in Late Arriving Rats (Group Mean ± S.D.) as a Function of Age and Cd Exposure

Weeks	Control	Group 2	Group 3	Group 4
1	4.94 ± 0.90	5.24 ± 0.77	4.19 ± 0.46	6.99 ± 1.35
5	19.87 ± 9.26	17.07 ± 2.28	14.06 ± 8.75	16.24 ± 4.64
9	25.30 ± 11.72	17.75 ± 2.94	15.97 ± 6.23	18.59 ± 6.44
16	22.69 ± 8.93	20.25 ± 4.08	21.93 ± 13.69	20.72 ± 5.59
28	20.99 ± 8.27	18.67 ± 2.13	24.12 ± 11.49	22.04 ± 6.68
37	26.93 ± 12.78	19.95 ± 6.55	22.00 ± 12.63	24.92 ± 3.88
50	25.16 ± 13.53	16.63 ± 3.81	19.12 ± 4.82	27.52 ± 11.42
70	22.34 ± 11.35	11.91 ± 5.05	16.72 ± 10.64	20.16 ± 5.51
84	17.41 ± 8.67	13.25 ± 10.60	15.02 ± 7.59	19.72 ± 8.82
96	18.11 ± 10.63	9.49 ± 1.57	13.84 ± 8.45	13.35 ± 2.89

Each value represents the mean of data from 4 rats.

See Table A-1 footnotes for an explanation of table construction and data analysis.

There were no significant differences between group means ($p \leq 0.05$) at any time point tested.

Table A-45. Group Mean Daily Urine Volume (ml/24 hrs) in Early Arriving Rats as a Function of Age and Cd Exposure

Weeks	Control	Group 2	Group 3	Group 4
1	8.80	10.00	7.70	7.60
5	13.02[x]	14.91[a]	10.28	5.29[a,x]
9	16.43	17.18	13.16	6.29
13	17.62	19.48	16.31	5.11
19	15.40	19.87	16.20	6.72
25	18.58	20.43[x]	19.30	8.53[x]
31	18.58	20.91	13.86	7.48
37	18.00	20.18[x]	13.44	8.23[x]
50	12.75	12.88	12.03	6.31
62	12.08	12.18	10.14	7.75
70	14.47	13.82	8.38	8.74
84	20.11	15.83	12.20	12.75
96	25.10	16.72	24.20	10.82
Overall	15.64[a]	16.47[b]	13.43[c]	7.87[a,b,c]

[a,b,c] Indicate that the differences between group mean values are significant at $p \leq 0.05$.

[x] Indicates that the differences between group mean values are significant at $p = 0.10$.

Each value represents the mean of data from 4 rats. The "overall" mean for each exposure group was calculated from the data for 52 rats.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-46. Group Mean Daily Urine Volume (ml/24 hours) in Late Arriving Rats as a Function of Age and Cd Exposure

Weeks	Control	Group 2	Group 3	Group 4
1	8.50	8.60	5.40	6.70
5	13.72[a]	11.77[b]	8.42	5.83[a,b]
9	20.60	18.21	8.65	6.31
16	19.20	16.30	9.60	6.40
28	14.70	17.40	7.80	6.90
37	12.69[x]	15.69[a,y]	8.48[y]	5.87[a,x]
50	8.83	10.19	5.98	4.71
62	8.68	13.45	5.30	4.89
70	9.21	16.03	7.04	5.40
84	14.75	14.23	10.65	10.40
96	9.00	12.97	7.58	12.11
Overall	13.06[a,c]	14.35[b,d]	8.27[c,d]	6.98[a,b]

[a,b,c] Denote that the differences between group means are significant at $p \leq 0.05$.

[x,y] Denote that the differences between group means are significant at $p = 0.10$.

Each value represents the mean of data from 4 rats. The "overall" mean for each exposure group was calculated from the data for 44 rats.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-47. Daily Excretion of Urine Total Protein (mg/24 hours) in Early Arriving Rats (Group Mean ± S.D.) as a Function of Age and Cd Exposure

Weeks	Control	Group 2	Group 3	Group 4
5	17.80 ± 3.16	16.70 ± 2.13	15.40 ± 1.91	17.50 ± 2.43
9	21.30 ± 4.67	21.10 ± 2.34	22.80 ± 3.25	20.60 ± 0.33
13	23.00 ± 4.42	21.80 ± 1.94	21.50 ± 2.45	26.40 ± 3.33
19	29.50 ± 7.52	29.10 ± 9.79	32.90 ± 10.40	28.30 ± 8.03
25	32.80 ± 14.99	30.80 ± 11.70	51.90 ± 31.40	33.80 ± 16.70
31	40.90 ± 20.50	34.30 ± 14.90	47.60 ± 23.30	37.40 ± 16.90
37	49.50 ± 26.00	51.10 ± 35.00	55.00 ± 30.30	63.00 ± 41.10
50	55.60 ± 29.30	69.90 ± 55.60	102.00 ± 72.10	75.20 ± 38.70
62	151.70 ±118.80	146.00 ±149.60	175.00 ±109.50	168.90 ±127.50
70	189.60 ±149.70	157.10 ±152.00	199.40 ±101.10	212.40 ±148.70
84	249.90 ±137.40	192.10 ±150.00	323.20 ±178.30	288.80 ±207.10
96	355.20 ± 84.10	234.90 ±165.90	338.10 ±216.20	410.00 ±164.20

Each value represents the mean of data from 4 rats.

Bartholomew's test for ordered means also was used for analyzing this data.

There were no significant differences between group means ($p \leq 0.05$) at any data collection point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-48. Daily Excretion of Urine Total Protein (mg/24 hours) in Late Arriving Rats (Group Mean ± S.D.) as a Function of Age and Cd Exposure

Weeks	Control	Group 2	Group 3	Group 4
1	3.43 ± 0.89	2.14 ± 0.28	1.95 ± 0.19	3.10 ± 0.90
5	18.10 ± 3.40	17.30 ± 1.87	12.60 ± 2.39	16.50 ± 0.72
9	18.60 ± 3.35	15.70 ± 2.19	13.90 ± 2.17	15.80 ± 2.26
16	22.50 ± 5.84	20.70 ± 5.35	18.00 ± 1.94	17.90 ± 0.79
21	21.00 ± 6.74	23.20 ± 5.96	18.30 ± 4.04	19.60 ± 4.46
28	22.30 ± 5.45	23.11 ± 5.84	17.50 ± 4.97	25.10 ± 10.40
37	24.40 ± 4.13	22.50 ± 2.54	23.70 ± 5.69	31.70 ± 19.40
50	31.20 ± 11.20	22.50 ± 5.45	18.10 ± 3.73	37.70 ± 24.50
62	44.20 ± 31.70	48.80 ± 50.20	27.90 ± 16.00	52.10 ± 36.60
70	55.00 ± 35.50	47.10 ± 37.40	44.10 ± 27.30	71.50 ± 28.40
84	76.30 ± 52.70	125.10 ±162.50	101.90 ± 73.70	95.10 ± 42.00
96	105.90 ± 31.50	172.60 ±117.70	159.80 ± 94.70	165.40 ± 68.00

Each value represents the mean of data from 4 rats.

Bartholomew's test for ordered means also was used for analyzing this data.

There were no significant differences between group means ($p \leq 0.05$) at any data collection point.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-49A. Mean pH of Urine from Early Arriving Rats as a Function of Age and Cd Exposure

Weeks	Control	Group 2	Group 3	Group 4
5	7.20	7.07	7.13	6.64
9	7.45	7.38	7.07	6.44
13	6.97	6.95	6.83	6.55
19	7.10	7.22	7.05	7.23
25	6.93	7.13	7.07	7.03
31	6.97	7.22	6.95	6.87
37	6.95	7.18	7.08	7.08
50	6.62	6.87	6.50	6.27
62	6.30	6.53	6.27	6.23
70	6.27	6.42	6.20	6.23
84	6.00	6.05	6.20	6.10
96	6.27	6.10	6.00	5.92
Overall	6.76	6.84[x]	6.71	6.55[x]

[x] Denotes that the differences between group means are significant at $p = 0.10$.

Each value represents the mean of data from 4 rats. The "overall" mean for each exposure group was calculated from the data for 48 rats.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-49B. Mean pH of Urine from Late Arriving Rats as a Function of Age and Cd Exposure

Weeks	Control	Group 2	Group 3	Group 4
5	7.17	7.58	7.23	6.93
9	7.02^{x}	7.23^{a,b}	6.70^{b}	6.55^{a,x}
16	7.25^{x}	7.30	6.87^{x}	6.80
28	6.93	7.13^{x}	6.90	6.58^{x}
37	6.87	7.18	6.63	6.70
50	6.55	6.47	6.13	6.28
62	6.33	6.15	6.00	6.05
70	6.37	6.38	6.04	6.03
84	6.30	6.20	6.05	6.25
96	6.03	6.09	5.96	6.03
Overall	6.70	6.82^{a,b}	6.50^{b}	6.48^{a}

[a,b,c] Denote that the differences between group means are significant at $p \leq 0.05$.

[x] Denotes that the difference between group means are significant at $p = 0.10$.

Each value represents the mean of data from 4 rats. The "overall" mean for each exposure group was calculated from the data for 40 rats.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-50A. Mean Specific Gravity of Urine from Early Arriving Rats as a Function of Age and Cd Exposure

Weeks	Control	Group 2	Group 3	Group 4
5	1.039	1.034	1.045	>1.050
9	1.035[a]	1.030[b]	1.046[x]	1.079[a,b,x]
13	1.029[a]	1.024[b]	1.044	1.070[a,b]
19	1.036[a]	1.026[b]	1.043[x]	1.070[a,b,x]
25	1.036[a]	1.031[b]	1.041[c]	1.066[a,b,c]
31	1.038[a]	1.027[b]	1.048	1.071[a,b]
37	1.041[a]	1.031[b]	1.050	1.071[a,b]
50	1.044[x]	1.037[a]	1.061	1.078[a,x]
62	1.051	1.042[a]	1.068	1.076[a]
70	1.040[a,b]	1.038[c,d]	1.069[a,c]	1.069[b,d]
84	1.035	1.041	1.056	1.051
96	1.050	1.028	1.030	1.047
Overall	1.039[c]	1.032[a,b]	1.049[a,c]	1.065[b,c]

[a,b,c] Denote that the differences between group means are significant at $p \leq 0.05$.

[x] Denotes that the difference between group means are significant at $p = 0.10$.

Each value represents the mean of data from 4 rats. The "overall" mean for each exposure group was calculated from the data of 48 rats.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-50B. Mean Specific Gravity of Urine from Late Arriving Rats as a Function of Age and Cd Exposure

Weeks	Control	Group 2	Group 3	Group 4
5	1.038	1.036	1.047	>1.050
9	1.034[a]	1.028[b,x]	1.051[x]	1.071[a,b]
16	1.034[a]	1.031[b,x]	1.050[x]	1.072[a,b]
28	1.042[a]	1.037[b]	1.054[c]	1.076[a,b,c]
37	1.049[a]	1.037[b,d]	1.058[c,d]	1.080[a,b,c]
50	1.059[a]	1.052[b]	1.068	1.085[a,b]
62	1.060[x]	1.046[a]	1.070	1.085[a,x]
70	1.060	1.043[x]	1.065	1.076[x]
84	1.046	1.047	1.054	1.063
96	1.045	1.040	1.058	1.055
Overall	1.046[d]	1.040[a,b]	1.056[a,d]	1.071[b,d]

[a,b,c,d] Denote that the differences between group means are significant at $p \leq 0.05$.

[x] Denotes that the differences between group means are significant at $p = 0.10$.

Each value represents the mean of data from 4 rats. The "overall" mean for each exposure group was calculated from the data of 40 rats.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-51A. Mean Left Kidney Weight, as a Percent of Body Weight as a Function of Age and Cd Exposure

Months	Control	Group 2	Group 3	Group 4
0	0.44	0.44	0.44	0.44
3	0.27[x]	0.32[x]	0.29	0.30
6	0.27[a]	0.29	0.29	0.31[a]
9	0.23[x]	0.25	0.27	0.27[x]
12	0.23[a]	0.24[b]	0.21[c]	0.28[a,b,c]
15	0.23[a]	0.24[x]	0.23[b]	0.29[a,b,x]
18	0.23	0.22	0.21[x]	0.26[x]
21	0.23	0.25	0.24	0.26
24	0.29	0.30	0.33	0.34
Overall	0.25[a]	0.26[y]	0.26[x]	0.29[a,x,y]

[a,b,c] Denote that the differences between group means are significant at $p \leq 0.05$.

[x,y] Denote that the differences between group means are significant at $p = 0.10$.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-51B. Mean Right Kidney Weight, as a Percent of Body Weight as a Function of Age and Cd Exposure

Months	Control	Group 2	Group 3	Group 4
0	0.57	0.57	0.57	0.57
3	0.34	0.36	0.36	0.38
6	0.26	0.30	0.28	0.30
9	0.20[a,x]	0.21[b]	0.24[x]	0.25[a,b]
12	0.22[a]	0.22[b]	0.19[c]	0.26[a,b,c]
15	0.21[x]	0.21[y]	0.21[z]	0.27[x,y,z]
18	0.21[a]	0.20[b]	0.19[c]	0.25[a,b,c]
21	0.22	0.22	0.22	0.25
24	0.27	0.29	0.31	0.31
Overall	0.24[a]	0.25	0.25	0.28[a]

[a,b,c] Denote that the differences between group means are significant at $p \leq 0.05$.

[x,y,z] Denote that the differences between group means are significant at $p = 0.10$.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-52A. Size of the Glomerulus, as Short and Long Diameters of Bowman's Capsule, as a Function of Age and Exposure to Cadmium (Group Mean, μm ± S.D.)

	0 Months	9 Months	15 Months	24 Months	Overall
Control:					
Short Diameter	70.0 ± 2.21	108.1 ± 10.77	116.0 ± 9.45	123.5 ± 9.97	115.5
Long Diameter	80.3 ± 5.58	126.6 ± 9.07	136.1 ± 11.30	145.4 ± 10.43	135.6
Group 2:					
Short Diameter	70.0 ± 2.21	102.7 ± 6.12	116.5 ± 5.95	118.9 ± 9.16	112.9
Long Diameter	80.3 ± 5.58	128.2 ± 7.78	138.1 ± 8.91	145.1 ± 15.10	137.2
Group 3:					
Short Diameter	70.0 ± 2.21	99.1 ± 5.09	110.3 ± 4.43	111.7 ± 8.05	106.8
Long Diameter	80.3 ± 5.58	120.8 ± 7.20	128.2 ± 11.30	131.5 ± 10.64[x]	126.6
Group 4:					
Short Diameter	70.0 ± 2.21	98.3 ± 11.70	120.0 ± 10.16	122.4 ± 6.26	113.3
Long Diameter	80.3 ± 5.58	117.8 ± 11.30	140.5 ± 13.10	148.5 ± 8.60[x]	135.4

[x] Denotes that the differences between the group means are significant at $p = 0.10$.

15 glomeruli were measured for each rat. Each value represents the mean dimensions of glomeruli from 7 to 8 rats.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-52B. Size of the Glomerulus, as Mean Diameter of Bowman's Capsule, as a Function of Age and Exposure to Cadmium (Group Mean, μm ± S.D.)

Months	Control	Group 2	Group 3	Group 4
0	75.1 ± 3.84	75.1 ± 3.84	75.1 ± 3.84	75.1 ± 3.84
9	117.3 ± 9.34	115.5 ± 5.73	109.9 ± 5.14	108.1 ± 11.30
15	126.0 ±10.19	127.3 ± 7.14	119.3 ± 7.50	130.2 ± 11.18
24	134.5 ± 9.69	132.1 ±11.94	121.7 ± 9.05[x]	135.5 ± 6.97[x]
Overall	125.6 ±11.66	125.1 ±10.77	116.7 ± 8.70	124.4 ± 15.57

[x] Denotes that the differences between the group means are significant at p = 0.10.

15 glomeruli were measured for each rat. Each value represents the mean of both the long and short dimensions of the glomeruli from 7 to 8 rats.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-53. Glomerular Volume Density (Vv) as a Function of Age and Cadmium Exposure

Months	Control	Group 2	Group 3	Group 4
0	0.091 ± 0.018	--	--	--
9	0.085 ± 0.012	0.091 ± 0.013	0.083 ± 0.018	0.080 ± 0.007
15	0.089 ± 0.016	0.083 ± 0.010	0.080 ± 0.013	0.089 ± 0.010
24	0.071 ± 0.013	0.053 ± 0.015[a]	0.058 ± 0.021[b]	0.088 ± 0.020[a,b]
Overall	0.082 ± 0.016	0.076 ± 0.020	0.074 ± 0.020	0.085 ± 0.014

[a,b] Denote that the differences between the group means are significant at $p \leq 0.05$.

$$Vv = \frac{\text{number of grid test points within the glomerular profiles}}{\text{total number of test points within the grid}}$$

Each value represents the mean ± S.D. of 7 to 8 rats.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-54. Glomerular Numerical Profile Density (Na) as a Function of Age and Cadmium Exposure

Months	Control	Group 2	Group 3	Group 4
0	2.84 ± 0.22	2.84 ± 0.22	2.84 ± 0.22	2.84 ± 0.22
9	1.83 ± 0.32	1.89 ± 0.16	1.72 ± 0.28	2.07 ± 0.42
15	1.71 ± 0.21	1.43 ± 0.54	1.49 ± 0.42	1.49 ± 0.23
24	1.68 ± 0.15	1.34 ± 0.32	1.46 ± 0.41	1.62 ± 0.38
Overall	1.74 ± 0.24	1.59 ± 0.33	1.60 ± 0.31	1.74 ± 0.43

Values are expressed as number of glomeruli/mm^2 of outer cortex and represent the mean ± S.D. of 7 to 8 rats. The "overall" mean for each exposure group was based on the data for 28 to 32 rats.

See Table A-1 footnotes for an explanation of table construction and data analysis.

There were no significant differences between group means ($p \leq 0.05$) at any sacrifice point.

Table A-55. Glomerular Numerical Density (Nv) as a Function of Age and Cadmium Exposure

Months	Control	Group 2	Group 3	Group 4
0	5.12	5.12	5.12	5.12
9	3.77 ± 0.54	3.69 ± 0.26	3.65 ± 0.47	4.17 ± 0.62
15	3.50 ± 0.35	3.38 ± 0.42	3.56 ± 0.34	3.17 ± 0.29
24	3.88 ± 0.38	3.84 ± 0.50	3.91 ± 0.66	3.37 ± 0.53
Overall	3.71 ± 0.45	3.63 ± 0.43	3.70 ± 0.50	3.59 ± 0.66

$Nv = (\frac{K}{B}) \cdot (\frac{Na^{2/3}}{Vv^{1/2}})$, with K = 1.00 and B = 1.38. Values are expressed as number of glomeruli/mm^3 of outer cortex and represent the mean ± S.D. of 7 to 8 rats. The "overall" means for each study group was based on the data of 28 to 32 rats.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-56. Group Mean Scores of Tissue Pathology as a Function of Age and Cadmium Exposure

	0 Months	9 Months	15 Months	24 Months
Inflammation:				
Control	0	1.56	2.44	3.21
Group 2	0	1.21	2.75	2.93
Group 3	0	1.44	1.81[a]	3.43
Group 4	0	1.94	3.50[a]	3.63
Interstitial Fibrosis:				
Control	0.25	2.00	3.19	3.43
Group 2	0.25	2.43	2.88	3.29
Group 3	0.25	2.44	2.50	3.71
Group 4	0.25	1.81	2.58	3.37
Glomerular Basement Membrane Thickening:				
Control	0	1.88	2.44	2.57
Group 2	0	1.86	2.50	2.71
Group 3	0	1.88	2.38	2.79
Group 4	0	1.56	2.26	2.50
Proximal Tubular Basement Membrane Thickening:				
Control	0	1.82	2.57	2.50
Group 2	0	2.00	2.50	2.21
Group 3	0	2.00	2.44	2.57
Group 4	0	1.69	2.26	2.63
Tubular Dilation/Casts:				
Control	0	1.38	2.81	2.93
Group 2	0	1.43	2.57	3.29
Group 3	0	1.69	2.06	3.21
Group 4	0	1.38	2.57	3.43
Hyperplasia:				
Control	0	0.13	1.68	2.43
Group 2	0	0.57	2.07	3.00
Group 3	0	0.94	1.63	3.36
Group 4	0	1.13	2.14	3.25

[a] Significant difference between group means at $p \leq 0.05$.

Tissue pathology was ranked as follows:

0 - 2	= mild changes
2.5 - 3.5	= moderate changes
4 - 5	= severe changes

Each value represents the mean score of 7 to 8 rats within a group.

Bartholomew's test for ordered means also was used for analyzing this data.

See Table A-1 footnotes for an explanation of table construction and data analysis.

Table A-57. Percentage of Individuals Having Mild, Moderate or Severe Renal Tissue Changes as a Function of Age and Cadmium Exposure

	9 Months			15 Months			24 Months		
	Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe
Inflammation:									
Control	7(87.5%)	1(12.5%)	0	5(62.5%)	2(25.0%)	1(12.5%)	2(28.6%)	2(28.6%)	3(42.9%)
Group 2	6(85.7%)	1(14.3%)	0	4(50.0%)	1(12.5%)	3(37.5%)	3(42.9%)	2(28.6%)	2(28.6%)
Group 3	8(100%)	0	0	5(62.5%)	3(37.5%)	0	1(14.3%)	2(28.6%)	4(57.1%)
Group 4	5(62.5%)	3(37.5%)	0	0	5(71.4%)	2(28.6%)	1(12.5%)	4(50.0%)	3(37.5%)
Interstitial Fibrosis:									
Control	6(75.0%)	2(25.0%)	0	3(37.5%)	3(37.5%)	2(25.0%)	1(14.3%)	3(42.9%)	3(42.9%)
Group 2	3(42.9%)	4(57.1%)	0	4(50.0%)	2(25.0%)	2(25.0%)	2(28.6%)	3(42.9%)	2(28.6%)
Group 3	3(37.5%)	5(62.5%)	0	4(50.0%)	4(50.0%)	0	1(14.3%)	3(42.9%)	3(42.9%)
Group 4	7(87.5%)	1(12.5%)	0	3(50.0%)	3(50.0%)	0	1(12.5%)	5(62.5%)	2(25.0%)
Glomerular Basement Membrane Thickening:									
Control	7(87.5%)	1(12.5%)	0	4(50.0%)	4(50.0%)	0	2(28.6%)	5(71.4%)	0
Group 2	7(100%)	0	0	2(25.0%)	6(75.0%)	0	2(28.6%)	4(57.1%)	1(14.3%)
Group 3	7(87.5%)	1(12.5%)	0	3(37.5%)	5(62.5%)	0	2(28.6%)	4(57.1%)	1(14.3%)
Group 4	8(100%)	0	0	3(42.9%)	4(57.1%)	0	4(50.0%)	3(37.5%)	1(12.5%)
Tubular Basement Membrane Thickening:									
Control	7(87.5%)	1(12.5%)	0	3(37.5%)	5(62.5%)	0	3(42.9%)	4(57.1%)	0
Group 2	6(85.7%)	1(14.3%)	0	2(25.0%)	6(75.0%)	0	3(42.9%)	3(42.9%)	1(14.3%)
Group 3	6(75.0%)	2(25.0%)	0	1(12.5%)	7(87.5%)	0	3(42.9%)	3(42.9%)	1(14.3%)
Group 4	7(87.5%)	1(12.5%)	0	3(42.9%)	4(57.1%)	0	3(37.5%)	5(62.5%)	0
Tubular Dilation/ Casts:									
Control	7(87.5%)	1(12.5%)	0	3(37.5%)	3(37.5%)	2(25.0%)	2(28.6%)	3(42.9%)	2(28.6%)
Group 2	6(85.7%)	1(14.3%)	0	3(37.5%)	3(37.5%)	2(25.0%)	3(42.9%)	2(28.6%)	2(28.6%)
Group 3	5(62.5%)	3(37.5%)	0	5(62.5%)	3(37.5%)	0	1(14.3%)	4(57.1%)	2(28.6%)
Group 4	7(87.5%)	1(12.5%)	0	2(28.6%)	5(71.4%)	0	1(12.5%)	5(62.5%)	2(25.0%)
Hyperplasia:									
Control	8(100%)	0	0	5(62.5%)	3(37.5%)	0	3(42.9%)	4(57.1%)	0
Group 2	7(100%)	0	0	4(57.1%)	2(28.6%)	1(14.3%)	3(42.9%)	2(28.6%)	2(28.6%)
Group 3	8(100%)	0	0	6(75.0%)	2(25.0%)	0	2(28.6%)	5(71.4%)	0
Group 4	7(87.5%)	1(12.5%)	0	5(71.4%)	2(28.6%)	0	2(25.0%)	4(50.0%)	2(25.0%)

The data are expressed as the number of rats within each group and the percentage of the group total having either mild, moderate, or severe tissue changes.

A Chi-square analysis also was used to analyze this data.

There were no significant differences between the groups ($p \leq 0.05$).

See Table A-1 footnotes for an explanation of table construction and data analysis.

TABLE B-1. PULMONARY FUNCTION - 12 MONTH GROUP

Dose	Date	Body wt., g	Lung wt., g	FVC/lung wt.	$FEV_{.15}$, %	V_{30}/body wt., %	V10, %	Intersections, no.
Control	12/01/80	656	2.32	5.5	79	3.51	80.4	53.1
	12/03/80	835	3.46	5.7	69	2.57	-	44.9
	12/15/80	648	1.80	7.0	84	2.26	81.9	44.2
	12/17/80	653	2.12	8.0	87	3.00	84.2	35.3
0.5 mg/l	12/02/80	755	2.35	8.5	63	-	-	43.1
	12/04/80	632	2.23	9.9	87	3.97	79.7	38.0
	12/16/80	718	2.19	7.4	88	2.62	85.1	46.9
	12/18/80	690	2.12	8.1	82	3.10	86.0	40.8
5.0 mg/l	12/02/80	833	2.29	7.1	87	2.23	80.6	49.1
	12/04/80	705	2.50	8.3	79	3.42	80.1	44.4
	12/16/80	773	1.92	8.3	87	2.38	86.4	47.5
	12/17/80	643	2.21	6.6	81	-	-	45.0
50 mg/l	12/01/80	640	2.11	7.1	78	3.47	82.4	51.0
	12/03/80	592	2.03	6.7	74	2.70	80.0	54.8
	12/15/80	569	1.92	6.9	51	2.60	82.1	45.0
	12/17/80	742	2.42	6.8	69	-	-	43.8

- no data

TABLE B-2. PULMONARY FUNCTION - 15 MONTH GROUP

Dose	Date	Body wt., g	Lung wt., g	FVC/lung wt.	FEV$_{.15}$, %	V$_{30}$/body wt., %	V$_{10}$, %	Intersections, no.
Control	03/09/81	972	2.53	9.53	70	2.57	-	29.9
	03/11/81	712	2.51	7.53	79	2.95	77.1	45.1
	03/24/81	920	2.25	7.08	80	2.17	-	42.3
	03/25/81	842	2.13	9.53	72	2.49	75.2	36.5
0.5 mg/l	03/10/81	807	2.34	7.86	91	2.35	77.9	44.8
	03/12/81	866	2.08	7.60	88	3.00	82.7	41.4
	03/23/81	853	2.08	8.17	81	2.47	78.2	41.3
	03/26/81	888	2.42	-	-	2.14	72.6	41.3
5.0 mg/l	03/10/81	736	2.12	7.69	87	2.45	77.8	53.1
	03/12/81	670	2.29	6.95	87	-	-	56.3
	03/23/81	933	2.16	-	-	1.97	76.6	42.8
	03/26/81	865	2.69	5.29	87	1.50	76.2	-
50 mg/l	03/09/81	638	2.18	8.85	90	3.45	77.3	54.1
	03/11/81	600	1.84	10.21	84	3.38	80.8	-
	03/24/81	646	2.25	6.90	85	2.63	81.2	-
	03/25/81	724	2.00	7.35	89	2.35	82.4	41.1

- no data

TABLE B-3. PULMONARY FUNCTION - 18 MONTH GROUP

Dose	Date	Body wt., g	Lung wt., g	FVC/lung wt.	$FEV_{.15}$, %	V_{30}/body wt., %	V_{10}, %	Intersections, no.
Control	06/01/81	862	2.46	7.02	76	2.37	83.3	37.6
	06/03/81	760	2.98	9.88	77	3.25	84.2	34.1
	06/16/81	850	1.96	6.98	84	2.06	85.7	35.9
	06/17/81	783	1.87	8.02	92	2.21	85.0	36.7
05. mg/1	06/02/81	957	2.14	9.16	55	2.59	86.7	28.7
	06/04/81	840	2.27	7.76	81	2.58	85.7	36.7
	06/15/81	815	2.08	5.87	83	1.83	83.9	34.9
	06/18/81	785	1.92	7.52	88	2.19	84.3	34.1
5.0 mg/1	06/02/81	935	-	-	58	3.28	87.9	26.5
	06/04/81	792	2.23	8.70	80	3.09	86.1	35.0
	06/15/81	610	1.79	6.88	78	3.16	82.9	38.5
	06/18/81	760	2.13	6.76	84	-	-	39.3
50 mg/1	06/01/81	682	1.98	8.64	86	3.02	84.5	34.5
	06/03/81	771	2.04	9.58	62	3.16	84.0	37.1
	06/16/81	926	2.02	8.67	79	2.30	85.9	31.0
	06/17/81	901	2.02	7.54	82	2.00	79.6	37.0

- no data

TABLE B-4. PULMONARY FUNCTION - 21 MONTH GROUP

Dose	Date	Body wt., g	Lung wt., g	FVC/lung wt.	$FEV_{.15}$, %	V_{30}/body wt., %	V_{10}, %	Intersections, no.
Control	08/24/81	930	2.18	8.1	78	2.37	82.7	34.4
	08/25/81	1302	2.49	10.3	61	2.04	82.6	37.5
	08/31/81	1034	2.35	8.2	77	1.76	88.5	34.6
	09/04/81	726	4.14	4.2	75	3.36	85.4	37.3
0.5 mg/l	08/26/81	927	2.44	8.2	75	2.79	86.3	36.4
	08/27/81	767	2.17	8.1	79	2.99	85.1	37.3
	09/01/81	895	1.71	10.1	72	2.61	87.4	40.4
	09/03/81	866	2.08	6.8	81	2.11	85.5	39.1
5.0 mg/l	08/26/81	908	2.53	6.5	61	2.73	83.1	37.6
	08/27/81	935	2.24	7.6	69	2.57	83.7	35.2
	09/01/81	815	1.82	8.7	65	2.43	83.6	43.2
	09/03/81	866	2.08	6.8	81	2.11	85.5	37.8
50 mg/l	08/24/81	837	2.32	8.4	80	2.99	85.2	41.1
	08/25/81	622	2.11	8.7	90	3.75	81.5	38.4
	08/31/81	912	2.08	6.3	89	1.62	81.8	41.5
	09/04/81	990	2.42	7.5	74	2.34	84.4	37.6

TABLE B-5. PULMONARY FUNCTION - 24 MONTH GROUP

Dose	Date	Body wt., g	Lung wt., g	FVC/lung wt.	$FEV_{.15}$, %	V_{30}/body wt., %	V_{10}, %	Intersections, no.
Control	12/03/81	550	2.12	6.3	86	3.65	81.6	*
	12/15/81	885	2.15	5.3	67	2.46	87.1	
	12/17/81	1120	2.40	5.6	71	2.04	85.1	
0.5 mg/1	11/30/81	673	2.02	6.0	85	3.22	78.8	
	12/02/81	759	2.04	6.5	75	3.04	87.0	
	12/14/81	672	2.05	6.0	84	3.96	89.8	
	12/16/81	816	1.95	7.4	87	2.65	85.6	
5.0 mg/1	11/30/81	628	2.29	6.3	84	4.25	84.6	
	12/02/81	540	-	-	84	3.89	85.7	
	12/14/81	787	1.85	6.3	64	2.10	83.0	
	12/16/81	865	2.02	6.9	81	2.14	80.5	
50 mg/1	12/01/81	625	2.04	6.6	72	4.30	87.0	
	12/03/81	625	2.04	6.0	86	3.30	80.1	
	12/15/81	665	1.87	6.8	87	2.81	85.6	
	12/17/81	587	1.69	8.7	93	3.42	86.1	

- no data

* data unavailable due technical problems